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# The Journal of Laboratory and Clinical Medicine

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## CLINICAL AND EXPERIMENTAL

### SERUM PROTEINS AND GLAUCOMA\*

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THE serum proteins play an important rôle in maintaining a fluid equilibrium between the blood and the tissues. Starling<sup>1</sup> showed that the osmotic pressure of the plasma crystalloids had little influence on the fluid distribution as compared with the plasma proteins, because the crystalloids easily pass through the vessel wall with the water. Govaerts<sup>2</sup> confirmed Starling's conclusion and proposed a theory to explain the interchange of fluid between the blood stream and the tissues. He demonstrated that the increase of the hydrostatic pressure or decrease of osmotic pressure of the serum proteins tends to cause transudation and to result in edema. Of the serum proteins, albumin with its smaller molecular weight showed a much greater osmotic pressure per gram than globulin. Therefore a decrease of albumin was more likely to cause edema than a deficiency of globulin.

The osmotic pressure of the blood proteins and crystalloids, and the capillary hydrostatic pressure are probably the chief factors which maintain the normal intraocular pressure. The osmotic pressure of the proteins has perhaps the most constant influence on the intraocular pressure. The hydrostatic pressure of the capillaries has not as yet been measured. There has been a tendency in the past to relate the hypertension in the eye with the lower osmotic pressure of the blood. Elliot<sup>3</sup> reported that Mayard observed 20 cases of glaucoma associated with a wave of epidemic dropsy. Hertel and Citron<sup>4</sup> believed that the total osmotic pressure of the blood of patients with glaucoma was generally less than that of normal persons. An osmotic pressure of over 6000 mm Hg was found in 16 per cent of the patients with glaucoma, and a pressure lower than 5600 mm Hg was never observed in cases with normal intraocular tension but

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was present in 45 per cent of glaucomatous cases Dieter<sup>5</sup> and Serr<sup>6</sup> observed no unusual deviation of the serum osmotic pressure in patients with glaucoma

*Methods*—The patients were allowed to become adjusted to the routine of the hospital. The blood for analysis was always obtained under the same conditions. It was drawn from the antecubital veins without stasis before breakfast after a fast of at least fourteen hours to avoid inspissation of the serum. The blood was collected and serum separated by the means which produced the least change of water content of the serum. The method of Howe as modified by Bruckman<sup>7</sup> was employed for the determination of plasma proteins. The ocular tension was taken by a standard Schiotz tonometer.

*Discussion*—The fractionation of the blood serum proteins of glaucomatous patients has not been reported previously. However, others have estimated the total serum proteins by the refractometric method which is known to give higher values than the Kjeldahl method.<sup>8</sup>

TABLE I  
SERUM PROTEINS OF GLAUCOMATOUS PATIENTS

NO	AGE	MEDICAL DIAGNOSIS	TYPE OF GLAUCOMA	★ TENSION SCHIÖTZ MM Hg		× NPN MG PER CENT	SERUM PROTEINS		
				RT	LT		TOTAL PROTEIN PER CENT	ALBUMIN PER CENT	GLOBULIN PER CENT
1	57	Normal	O U Chronic	26	47	28	6.08	4.26	1.82
2	76	Arteriosclerosis	O U Chronic	34	29	34	6.20	4.37	1.83
3	62	Normal	O U Chronic	65	32	41	6.20	4.46	1.74
4	57	Ing. hernia	O U Chronic	26	25	27	6.24	4.47	1.77
5	58	Arteriosclerosis	O U Chronic	29	40	29	6.81	4.35	2.46
6	76	Ing. hernia, arteriosclerosis	O U Chronic	50	31	29	7.04	4.25	2.79
7	62	Ing. hernia	O D Anophthalmos	—	61	33	7.05	4.61	2.44
			O S Chronic						
8	58	Normal	O U Chronic	42	45	28	7.07	4.56	2.51
9	69	Hypertension	O U Chronic	33	27	31	7.07	4.73	2.34
10	65	Hypertension, arteriosclerosis	O U Chronic	29	52	33	7.13	4.22	2.91
11	51	Hypertension	O U Chronic	26	28	29	7.14	5.90	1.24
12	64	Hypertension	O U Chronic	26	38	31	7.18	4.28	2.90
13	55	Normal	O U Chronic	51	32	27	7.27	5.21	2.06
14	82	Arteriosclerosis	O U Chronic	74	25	34	7.30	4.73	2.57
15	47	Normal	O U Chronic	90	70	24	7.36	4.88	2.48
16	59	Syphilis	O D Chronic	25	51	28	7.36	5.02	2.34
			O S Acute						
17	69	Hypertension, arteriosclerosis	O U Chronic	43	25	29	7.45	4.52	2.93
18	58	Normal	O U Chronic	61	29	28	7.61	4.74	2.87
19	63	Hypertension, arteriosclerosis	O U Chronic	36	28	22	7.99	5.01	2.98
20	73	Hypertension, arteriosclerosis	O D Phthisis bulbi	—	65	35	8.00	5.04	2.96
			O S Chronic						

The value of the serum proteins in glaucomatous patients has a fairly wide range. Repeated determinations the following day showed practically little difference. The total serum proteins varied from (see Table I) 6.08 to 8.00 per

cent, the serum albumin from 4.22 to 5.90 per cent, and the serum globulin from 1.24 to 2.98 per cent. If Bruckman's determinations are accepted as normal values, the serum proteins fall within the normal range. From these data there is no reason to believe that chronic glaucoma is directly associated with the concentration of blood proteins.

*Conclusion*—There is no essential quantitative difference between the serum proteins of healthy persons and of patients suffering with an uncomplicated glaucoma.

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### THE NONGLUCOSE REDUCING BODIES IN DIABETIC BLOOD\*

RAWSON J. PICKARD, M.D., AND FRANCIS W. GODWIN, SAN DIEGO, CALIF.

THE nonglucose reducing body which makes the reduction with most blood sugar methods too high has already been found constant in health and disease, and specifically in diabetes, by Folm and Svedberg.<sup>1</sup> This paper confirms their report. We estimated the substance in terms of glucose as the non-fermented reduction by the Folm-Wu method after treatment of the whole blood with an equal quantity of yeast suspension for five minutes. This substance, called "glucid X" by Fontes and Thivolle,<sup>2</sup> the "saccharoid" of Benedict,<sup>3</sup> is the reducing substance analysed by Best,<sup>4</sup> in part composed of pentose which makes the apparent blood sugar by the Folm-Wu method about 20 mg per cent too high. The amount of true glucose is obtained by subtracting the value for glucid X from the apparent glucose, or the true glucose may be determined directly by the Folm-Wu method on the copper filtrate. Somogyi suggests<sup>5</sup> and glucid X estimated as the excess reduction in the tungstate filtrate. As a rule we found the two methods checked closely. It is possible that the copper and tungstate comparison may prove to be the better, since glucid X is sometimes completely fermented in a few minutes (vide infra) and may not infrequently be destroyed in part by five minutes' yeast fermentation.

Our chief interest was in the "Y-reduction" which Pickard, Pierce and associates<sup>6</sup> found constantly as a reduction over that of glucose and glucid X

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TABLE I  
GLUCOSE, GLUCID X AND Y REDUCTION (GLUTATHION?) IN DIABETES

PATIENT	FW	ION	GLUCOSE	GLUCID X	Y REDUCTION
1 Re, Dr Oatman Sugar free, diet only 3 yr	156	210	140	16	54
Same, later	132	210	116	16	78
2 Ma, Dr Potter No insulin	490	550	484	6	60
3 Br, Dr Potter	139	206	115	24	67
4 Fa, Dr Brenner Diet only Age 75	178	208	157	21	30
5 Ju 31, Mercv H	244	320	221	23	76
6 Be, Dr Miller	170	234	144	26	64
7 Ho, Dr Brenner Blood stood 16 hr in room	284	290	276	8	6
Same, after 10 U insulin	265	298	253	12	33
8 Bg, Dr Miller	400	550	385	15	150
4 d later, 2 hr after 40 U insulin In shock Blood stood room 16 hr	21	106		8	85
7 d later, on 20 U daily, blood 2 hr after insulin	60	128	44	16	68
9 d later, 40 U daily	122	180	106	16	58
11 d later, same insulin Urine, trace sugar	160	185	140	20	25
2 wk later, same dosage	196	250	186	10	54
9 d later, same dosage	261	319	225	34	58
3 wk later Has gained 15 lbs Urine trace sugar	135	170			35
9 Dr, Dr Miller Diet only	128	206	116	12	78
3 wk later	120	152	113	7	32
10 Me, Dr Brenner Age 60, diet only was 480 glucose	206	198	178	28	0
Later date	280	350			70
11 Fo, Dr Miller Diet only, ins refused	348	390	335	13	42
12 Ha, Dr Brenner Diet, no insulin	140	198	127	14	58
14 De, Dr Kennell Diet only, 3 yr sugar free urine	150	250	130	20	100
15 38 178 Dr Ball Insulin 2 hr previously	304	300	280	20	0
16 38 181 Dr Ball Insulin 3 hr previously	325	355	310	15	30
Rabbit, 2.5 kg	127	200	99	28	73
Same, next day, before insulin	105	240	90	15	135
1/4 hr after 15 U insulin	55	145	41	15	90
FW, apparent sugar by Folin Wu method Ion, same by Ionesco method Glucose, true glucose, after subtracting glucid X from FW All figures mg per 100 c c					

Pickard<sup>7</sup> has shown that the Y reduction is probably due in large part to the glutathione of the cells. We estimated the Y reduction by subtracting the reduction by the Folin Wu method (glucose plus glucid X) from the reduction as glucose, in the same tungstate filtrate, by the Ionesco method.<sup>8</sup> The Y reduction in normal young people was 40 mg per cent average,<sup>9</sup> in normal adults it was the same.<sup>7</sup> The average Y-reduction in 26 diabetic blood specimens was 54 mg., varying in a wide range as was also the case in normal blood specimens. In the diabetic glutathion as an oxygen carrier in the cells might be influenced by the excess glucose in the blood. We found a definite increase in the Y-reduction on the average. Two samples, however, showed no Y-reduction. In case 8 with the highest figure, 150 mg., the Y-reduction became lower with improvement in her condition, and gain in weight, the average of her last 4 examinations is normal.

Since Fontès and Thivolle found no reduction in the tungstate filtrate after fermentation of the blood with yeast, using their reagents, they thought that glucid X was always fermentable completely and immediately with the true glucose. The loss of sensitivity of their reagents to glucid X after fermentation shows evidence of a change in this substance preliminary to its destruction. Most writers describe glucid X as the "non-fermentable" non glucose reduction. Pickard, Pierce and associates found glucid X is occasionally fermented with the true glucose, giving no reduction with the Folin-Wu method, in other bloods it was fermented completely in an hour. We have found that it completely disappears after three days' fermentation (Table II).

TABLE II  
FERMENTABILITY OF GLUCID X, "NON-FERMENTABLE," NONGLUCOSE REDUCING BODY

PATIENT, TABLE I	TIME OF FERMENTATION			REDUCTION MG PER CENT AS GLUCOSE			
	5 MIN	2 HR	16 HR	24 HR	2 DA	3 DA	4 DA
3	24			13			
5	23	16					
8	15		10				
10	28						0
6	26						1
7	12				3		
8	16				3		
St	12			9			
14	20					0	
13	15					0	
11	13				6		

Equal quantities of blood and yeast (Fleischmann) suspension    Room temperature    Ordinary tungstate precipitation, Folin Wu blood glucose technique

Besides its fermentability Fontès and Thivolle found glucid X decreased after insulin and in ratio with the glucose. They thought therefore it should continue to be estimated with the blood glucose as a physiologically similar sugar. Table I does not show any relationship of either glucid X or the Y-reduction to the amount of glucose. In 24 blood samples glucid X averaged 16.5 mg per cent, the normal by the Folin-Wu technique is 20 mg., and Folin and Svedberg found the

same in diabetics. The lowest figure we found for glucid X, 6 mg is in the blood with the highest glucose. The two bloods in which glucid X was 8 mg had stood at room temperature for sixteen hours, and we have found that autolysis on standing destroys glucid X in part, as it does the glucose. Glucid X was low in patients who never had insulin.

Since the amount of glucid X does not vary much and in any case is small compared to glucose, there has been little practical result from the confusion in the "normal" figure for blood sugar which has resulted from the introduction of various new methods for glucose, methods which have only approximated the true values. The Folin ferricyanide and the Benedict copper techniques give about half the value for glucid X of the Folin-Wu or the Hagedorn-Jensen, perhaps showing only the pentose fraction. We believe that it is desirable to make the estimation of blood glucose exact, now that there is a simple, practical and accurate technique. This technique is the precipitation of the proteins, and of glucid X, by copper, as Somogyi suggests, followed by the Folin-Wu blood sugar method.

1 The substances causing the Y-reduction are increased over the normal in diabetic blood, but vary widely.

2 The "non-fermentable" glucid X is fermented, completely in three days occasionally it is destroyed with the glucose.

#### TECHNIC

*Copper Precipitation*—One volume blood, take in 7 volumes water, add 1 volume 7 per cent copper sulphate, shake. Add 1 volume 10 per cent sodium tungstate drop by drop, shaking. Filter.

*Ionesco Method*—Put 5 c c 1/10 blood filtrate in a 200 by 15 tube, add 2 c c N/1 NaOH, 1 c c ferricyanide reagent (23 gm K ferricyanide, 23 gm KOH, water to 1000), 10 c c water. Boiling water-bath twelve minutes, cool, color should be yellow. If colorless repeat using 2.5 c c filtrate. Add 5 c c 20 per cent Fe free  $\text{H}_2\text{SO}_4$ . Titrate from microburette with N/60  $\text{KMnO}_4$  to rose color. Subtract correction of blank. One c c N/60  $\text{KMnO}_4$  equivalent to 100 mg glucose per 100 c c blood.

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# ANGIONEUROTIC EDEMA ITS RELATION TO BACTERIAL HYPERSENSITIVITY\*

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THE suggestion is to be found in most recent discussions of the disease that the peculiar symptom complex described under the name angioneurotic edema may be included with those phenomena which we crowd under the ever-enlarging canopy of the term allergy. For the most part it is seldom more than a suggestion and definite evidence of a convincing character demonstrating sensitization and the amelioration of symptoms following desensitization is largely lacking. Quincke, whose name has been given to the condition, is responsible for the opinion that the fundamental pathogenic factor is an angioneurosis, whatever that may be. This belief has persisted and would seem to us to have exerted a pernicious influence in inhibiting active inquiry regarding the actual cause. Though the clinical syndrome is a fairly common one and is now generally recognized there has been astonishingly little curiosity concerning its odd manifestations, if we may be guided by the reported studies. Nor can the usual benign character of the disease be accepted as an excuse for this apparent lack of interest because no one can witness the picture of acute suffocation subsequent to edema of the glottis without realizing the frightful potentialities involved whenever the diagnosis of angioneurotic edema is made.

The obvious relationship between the disease and giant urticaria has long pointed in the direction of specific hypersensitivity, and Osler<sup>1</sup> mentions one case in which desensitization with peptone was followed by the relief of symptoms. Mackenzie,<sup>2</sup> in his discussion of this disease, writes as follows: "Recent evidence makes it probable that in some of the nonhereditary cases the condition is due to food allergy." He then drops this phase of the subject without further comment. Wherry,<sup>3</sup> who has been the first to make so many important suggestions, was, we believe, the first to point out the probable causal relationship between bacterial hypersensitivity and angioneurotic edema. Unfortunately, his patient died of an intercurrent infection before he was able to complete the process of desensitization. The evidence he offers, however, is most interesting and it was the stimulation of Wherry's report which directed us to the careful study of a series of cases of angioneurotic edema.

We wish to report in this paper the detailed study of three such cases. These three do not exhaust our series but we prefer to report them in considerable detail and this would be impossible in a larger series. Also the three patients under consideration have all been observed for a period of at least two years *since* desensitization, and we feel that this is the minimal time factor which

\*From the Department of Internal Medicine and the Louis Kuhn Diagnostic Laboratory.  
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justifies the drawing of conclusions. All the cases to be reported are of the non-hereditary type and it may be possible that such conclusions as we have reached will obtain only in that form of the disease, though Wherry's case had a decided hereditary background.

This study forms only one small phase of a much more comprehensive inquiry into the field of bacterial hypersensitivity, and it will be necessary to refer very briefly to certain concepts and methods in technique which are more fully elaborated in previous papers<sup>4 5 6 7</sup>. Any one interested in the application of our methods is referred to these reports. We have been especially interested in the possible significance of intradermal tests when employed to indicate specific sensitivity to bacterial antigens. We believe that we have been able to use such tests to great advantage in studying bacterial asthma, arthritis, nonulcerative colitis, and certain skin conditions, and by use of them have demonstrated in such conditions a fundamental bacterial hypersensitivity. Antigens of the bacterial type, selected on the basis of positive skin reactions and given by the "desensitization" method have yielded excellent therapeutic results.

Interested in such problems, it was inevitable that we should include in our studies such a curious disease as angioneurotic edema. We began these studies by attempting to demonstrate the role of food allergy in the etiology of the disease. This was done by the use of intradermal food tests and of "selective restriction" diets. By these means we were able to obtain evidence suggesting food sensitization in two cases and were led to a striking therapeutic result in the management of one of them. In a disappointingly large number, however, we were unable to prove the existence of food allergy, and utilized the methods which we had developed for the study of bacterial hypersensitivity for the further investigation of these refractory cases. It is this second group which we wish to consider in the present paper.

CASE 1—This patient was a white male, aged thirty eight, and he gave a history of unusually good health prior to an acute attack of sinusitis which occurred in 1924. We first saw him in October of 1928. He had been having recurring attacks of angioneurotic edema since the spring of 1925. The attacks were rather mild and occurred at two to three month intervals but they almost invariably involved the face, and the patient was as much distressed by the cosmetic effect as by the actual discomfort. There was also a history of fleeting attacks of "hives" but none of these had lasted longer than from twelve to eighteen hours.

Food tests showed him to be sensitive to cocoa and certain sea foods. These had never constituted an important part of his diet and were eliminated with no apparent therapeutic result. We then directed our attention to the problem of bacterial sensitization. Enteric cultures yielded a number of organisms but he failed to show marked sensitivity to any of them. According to his history the disease had its onset following an acute attack of sinusitis and the x-ray examination of the sinuses suggested a chronic ethmoid infection. There was also a history of postnasal "drip" and headache, both of which postdated the attack of sinusitis.

Cultures were made from material obtained by suction from the ethmoid region. These cultures yielded a pure growth of hemolytic staphylococcus albus. An antigen prepared from this strain produced a marked local reaction when injected into the skin. Desensitization was carried out beginning with one minim of the vaccine in dilute suspension and slowly increasing the dose by one half minim intervals to a maximum dose of four minims. Injections were given every second day and were continued for six months. Before the first month had passed he had one mild attack and to date (December, 1931) he has never had

another. This patient responded more readily than any other we have studied and again emphasizes a fact which we have previously pointed out that the hemolytic staphylococcus is an excellent antigen for use in specific desensitization. There was also marked improvement in the symptoms of chronic sinusitis.

CASE 2—A white woman of forty five whose history of urticaria and angioneurotic edema was of six years' duration. The disease most frequently exhibited itself as a giant urticaria but upon frequent occasions there was swelling of the ankles and feet and occasionally of the lips. As there was no financial handicap to the search for relief this patient had been studied in several clinics of distinction both in the east and in the middle west. Dietary restrictions had been attempted but had given no permanent relief and "food tests" had been carried out on several occasions but had failed to give information of value in treatment. The attacks were, in general, cyclic with excessive fatigue, nervousness and excitement acting as precipitating factors. Following complete rest and a change of environment there had been one distinct intermission of months during which she was practically symptom free. This was followed by a exacerbation of symptoms more exaggerated than ever and when we first interviewed the patient she was suffering with almost daily attacks of urticaria and frequent seizures of the true angioneurotic type.

In our study of the problem we could make no case for the probability of a food sensitization. We reviewed the previous careful studies and these as well as our own investigations failed to convince us that we were dealing with food allergy. The possibility of a bacterial sensitization of the enteric type was suggested by both the history and the physical examinations. The history was that so often given by patients with an "irritable colon," a condition which we have previously discussed in its relationship to bacterial hypersensitivity. Upon examination the distal colon was distinctly spastic and tender, the proximal colon definitely distended. Hence, we directed our attention to the study of the intestinal flora.

Stool cultures yielded the following organisms: (1) *B. coli*, strongly hemolytic, (2) *B. coli*, nonhemolytic, (3) *B. mucosus capsulatus*, (4) streptococcus of indifferent cultural reactions, and (5) *Staphylococcus albus*, nonhemolytic. The predominating organism in the fecal flora, however, was the hemolytic colon bacillus and the lysis shown when this organism was grown upon blood agar plates was remarkable. Intradermal tests were run using each of the five strains as antigens. The hemolytic *B. coli* gave rise to a marked positive reaction with erythema and edema at the site of injection. There were very moderate reactions to the nonhemolytic colon bacillus and to *B. mucosus capsulatus* while there was no reaction to the other strains. We selected strain 1 as that most distinctly indicated by the skin reactions and further tested the causal relationship of this organism by giving a two minim intradermal dose of an autolysed filtrate. This proved to be a most interesting and enlightening experiment, for within two minutes of the injection there was marked local edema and within five minutes the patient complained of numbness and tingling of the upper lip and, under our eyes, developed a typical angioneurotic swelling of the upper lip and right cheek. This was controlled with adrenalin. Here we were able to produce not only local swelling by the intracutaneous injection of an autolysed antigen, but what is most convincing, a focal reaction as well.

The treatment consisted in desensitization by using heat killed suspension of the hemolytic *B. coli* as the antigen. A dilute saline suspension was employed and the treatment started with the intracutaneous injection of one minim. This dose was continued every second day until eight injections had been given and was then increased to one and one half minims. From this point the dosage was slowly advanced by one half minim intervals and each interval was maintained for from four to six injections. Five minims was the maximum dose and when reached, was continued. The vaccine was given over a period of three and one half months and then discontinued. During the first two weeks of the treatment there were two mild attacks, then freedom for five weeks, the longest period without an attack in months. There was then a rather sharp attack with swelling of the face which followed an attempt to advance the dose of vaccine too rapidly. This was readjusted and the process of desensitization continued as outlined. From this time on the symptoms diminished in severity very rapidly. There were scattered mild attacks

of urticaria but no major swelling and after a few months these stopped entirely. After four months of treatment the skin sensitivity had practically disappeared. A year later the patient was retested with the antigen and found to be again moderately sensitive, so she was given two minims of vaccine every second day for two months. During this interval of secondary treatment there were no skin symptoms and when tested for sensitivity nine months later the reaction was negligible. She has now been free of urticaria and angioneurotic edema for more than two years.

**CASE 3**—This patient was a white woman of forty who presented the usual symptoms of swelling of the face, hands, and feet which came on rapidly, lasted about twenty-four hours and then, as suddenly, disappeared. On several occasions she had experienced severe gastroenteric crises which were associated with the attacks. Previous to the actual swelling there would often be premonitory symptoms consisting in a feeling of distinct warmth and itching in the particular area where the swelling was to appear. It is interesting to note that this patient gave a history of asthmatic attacks which followed influenza in 1918 and which, apparently, were self-limited since they ceased within two years and have not recurred.

The initial attack of angioneurotic edema occurred early in 1925 and came on after a prolonged exposure to water and cold. We first saw the patient in December of 1927 so the condition had been established for almost three years. At first the attacks had been infrequent but after the first year the interval of remission had shortened rapidly and two or three attacks each month had been the story for the last twelve months before we studied her case.

The general physical examination was essentially negative with the exception of a distinctly spastic distal colon and she gave a history of chronic constipation of years' duration. When studied for sensitivity to food proteins she was found to react to egg albumen, milk, fish, and nuts. Elimination of these foods from her diet did not in any way influence the course of her disease.

From her stool cultures we recovered a *Staphylococcus aureus* and *Streptococcus hemolyticus*, *B. cloacae*, and *B. coli communior*. When skin tested to these various strains in pure culture, she was markedly sensitive to the *B. coli communior*. Similarly tested to a nonautogenous strain of the same organism with which we happened to be working at the time, she gave a mild reaction in no way comparable with that caused by the autogenous strain. Treatment with the "active strain" was started in January of 1928. The first injection was given employing one minim of a fairly heavy suspension and was immediately followed by an acute attack of angioneurotic edema. This necessitated a dilution of 1:5 and she tolerated a one minim dose of this suspension without untoward results. We were able to continue with this dose, giving it every second day, for three weeks. Increased dosage, at this point, again brought on fresh manifestations and during an interval allowed to pass while the vaccine was being further diluted, the patient had a spontaneous attack associated with acute edema of the glottis which necessitated hospitalization and the closest observation. We mention this fact because certain writers have been inclined to consider the nonhereditary form of the disease as benign and to associate edema of the glottis with the hereditary type alone. Such a point of view is contrary to fact, and we would emphasize the statement that edema of the glottis may occur in either type.

Desensitization was resumed using very minute doses of a quite dilute suspension and after several weeks we were able to increase the dose cautiously without reactions. The process was a tedious one but gradually the attacks diminished in severity and intensity. In June, 1928, a fresh specimen of stool yielded the same organism and in addition a strain of *B. mucosus capsulatus* to which the patient was also quite sensitive. This was added to the vaccine and the desensitization resumed, the maximum dose employed being four minims. She continued to have mild attacks through the early fall of 1928 but was so encouraged by the distinct change in the character of the seizures that she cooperated willingly in the tedious treatment. Her skin sensitivity was at this time markedly decreased and through the following winter she was almost free from symptoms. Treatment was continued until August of 1929 and then she was discharged and told to return in

the event of a recurrence of her former trouble. She was last interviewed in November, 1931, two and one half years since her last mild attack and there have been no recurrences.

#### DISCUSSION

We have presented in considerable detail a study of three cases of angioneurotic edema in which we were unable to demonstrate the causal relationship of food sensitization. We were able in each case to show what we have come to regard as definite evidence of bacterial hypersensitivity and by an adequate course of desensitization in which selected antigens were used we have kept each of these patients symptom-free for more than two years. The first case presented a rather simple problem and the course of desensitization was not an arduous one. Cases 2 and 3 on the other hand illustrate very well the difficulties encountered when one attempts to desensitize susceptible patients with bacterial antigens. At the same time they demonstrate the value of careful, persistent effort in this direction. We have previously called attention to the great value of vaccine therapy when conducted in this manner and wish again to emphasize the fact that desensitization of an already sensitized individual is one problem and the immunization of a noninfected individual is distinctly another. We are certain that had vaccine been given to these individuals in massive doses they would not have improved rather the converse would have resulted. We have seen the exaggeration of symptoms follow the use of large doses of vaccine not only in angioneurotic edema but also in asthma, nonulcerative colitis and a certain type of joint effusion. We are confident that the failure to take this factor into account is responsible for many of the failures in vaccine therapy. The other great source of disappointment is the failure to select "specific" autogenous antigens to which the patient is sensitive.

#### CONCLUSION

We believe that we have demonstrated the importance of bacterial hypersensitivity as an etiologic factor in angioneurotic edema.

Adequate desensitization is associated with the disappearance of symptoms.

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## THE ALLEGED CLINICAL INTESTINAL ACTIONS OF APOCODEINE\*

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IT HAS been reported that apocodeine is an efficient and valuable clinical cathartic, which can act orally and hypodermically. The claims for the cathartic action appear to be founded on certain experimental results which seemed to show that the action of apocodeine is mediated through depression or paralysis of the sympathetic ganglia, thus leaving the intestine to parasympathetic nerve control. If true, the advantages of such a drug would be obvious in conditions accompanied by over-stimulation of the sympathetic nervous system, as in the so-called sympathicotonia and related nervous disorders, in post-operative paralytic ileus, in the paresis of peritonitis, etc.

Perhaps the last, and also most convincing, clinical report is that of W C Alvarez,<sup>1</sup> who reported that the oral administration of apocodeine in doses of 1/5 to 1/10 gr (44 to 65 mg) orally caused one to three normal evacuations of the bowels daily, generally, the drug was given together with atropine. Some of Alvarez's patients continued the use of apocodeine for as long as three years; there was neither habituation, nor need to increase the dosage. Several years previously, Gumard,<sup>2</sup> Combemale<sup>3</sup> and Toy<sup>4</sup> all reported favorably upon the cathartic action of apocodeine. The two former reporters used the drug hypodermically, the latter employed it orally as a sedative for psychotic patients and observed the cathartic action only incidentally.

The only other clinical use claimed for apocodeine is as an expectorant, reported by Murrell<sup>5</sup> in 1871, and by Frohner<sup>6</sup> in 1893. This could be the result of a nauseant action, not infrequently caused by the apocodeine, which chemically resembles apomorphine. Our own experiences with apocodeine agree with the claims of Legg,<sup>7</sup> who, in 1870, found no effect on man using the drug in doses ranging from 1 to 3 gr (65 to 195 mg).

Concurrently with our clinical trials, Dr E W Schwartze<sup>8</sup> reinvestigated the important pharmacologic actions of apocodeine. Schwartze was unable to confirm the claim that apocodeine acts as a paralyzant for sympathetic ganglia of the gastrointestinal and circulatory systems. Excised intestinal musculature was depressed by the drug, and the cathartic action in intact animals was found to be "variable and indefinite." He failed to observe any erection of the hairs or redness and edema of the face, although the doses of apocodeine employed were sufficient to cause considerable circulatory depression. A certain, though fleeting, increase in intestinal peristalsis accompanied the circulatory depression, but only at the expense of such depression, and the intestinal action could be recapitulated with a circulatory depression of mechanical origin. Emesis and defecation occurred in dogs in about the same way as after morphine, the seat of these actions was presumably central, and not ganglionic or in the intestine.

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Schwartz could demonstrate a paralytic, nicotine-like action of the apocodeine on the ganglia of the cardiac vagi, but this is irrelevant to a consideration of the intestinal and cathartic actions

*Methods*—Apocodeine hydrochloride was used by us in a total of 22 patients. Three of these patients suffered from postoperative ileus. The remaining 19 patients had apparently normal gastrointestinal tracts, four had entered the hospital for cystoscopy and pyelograms, one came in for dilatation of the ureters, one had pyelitis, and the remaining 16 were normal obstetric patients one week postpartum. All the patients were confined in bed at the time of our investigation. The apocodeine was used in aqueous solution given intramuscularly in the gluteal region.

The doses of apocodeine in our patients varied from 12 to 97 mg, and the same three products of the drug used by Dr. Schwartz in his pharmacologic investigation were used by us. Two of these samples of apocodeine (Merck) were dark green in color, amorphous and presumably impure. One other sample was specially prepared for our use by Merck and Company, it was white, amorphous and presumably purer than the green products.

Control readings of blood pressure, pulse and respiration were taken at fifteen-minute intervals during one hour before the administration of the drug and during three hours afterwards. The patients were closely observed for changes in diameter of the pupils, for presence of colic, nausea and defecation, for relief of pain where pain was present, and for subjective symptoms. The data obtained were tabulated and summarized, but the table is omitted as the net result for our purpose was practically negative, an abstract of the essential results is here presented.

*General Effects*—In two (9 per cent) patients (Vr and Rs) slight constriction of the pupils occurred. Colic of slight and transient character occurred in one (4.5 per cent) patient (Hh). Nausea occurred in 6 patients (Hh, Wd, Li, Rs, Ly and Kg) or 27.2 per cent, and of this number 4, or about 66 per cent (Li, Rs, Ly and Kg), vomited. Defecation did not occur in any of the patients. In three patients (Cr, Ht and Rs), a slight narcotic action with relief of pain was observed. In two patients (Ge and Rs), who had severe pain following ureteral catheterization, the apocodeine failed to give any relief after three hours, and morphine sulphate had to be used.

All three samples of apocodeine caused more or less local irritation after intramuscular injection. The irritation after the dark green apocodeine was comparatively negligible but the white and supposedly purer product gave such severe local reactions that its use was discontinued. The injection areas of the three patients treated with white apocodeine threatened to break down and form abscesses and all three patients experienced systemic reactions, including fever up to 38° C.

*Circulatory Effects*—In no patient did the apocodeine produce any notable change in blood pressure, pulse rate or respiration. The circulatory depression commonly observed in laboratory animals did not occur, probably because the dosage of the drug was not sufficient, and it was not used intravenously as was the case in animals. The widest variation in blood pressure from the mean was 5 per cent and that in the pulse 7 per cent.

*Failure of Apocodeine in Postoperative Ileus*—The action of apocodeine was closely followed in three patients with paralytic ileus. Patient Wo developed ileus following a hysterectomy and appendectomy, forty-five minutes after the injection of 16 mg apocodeine the patient complained of nausea, but did not vomit. Five hours after the injection there had been no colic, and no passage of flatus or feces. At this time, 40 cc of 20 per cent sodium chloride solution were given intravenously and soon afterward the patient passed flatus, and peristaltic sounds were audible through the abdominal wall. Patient Ly, who suffered a partial ileus as the result of a plevitis, received two doses of apocodeine (11 mg each), given four hours apart and a third dose of 16 mg eight hours after the second dose. The patient was nauseated and vomited after the second dose. There was no improvement in the ileus until thirty-four hours after the last dose of apocodeine, that is long after its use had been abandoned, and other measures adopted. Patient Kg developed ileus following the operative relief of an intestinal obstruction. She was given two doses of apocodeine (each 11 mg) within six hours. There was considerable nausea and the patient vomited twice, but there was no colic, or passage of flatus or feces. The ileus was overcome forty-eight hours later by vigorous treatment of the patient with hypertonic sodium chloride solution intravenously and pituitary extract hypodermically.

*Comment*—In our hands three specimens of apocodeine did not exert any demonstrable clinical effects on the intestine, as indicated by passage of flatus and feces in 22 patients. There were no cathartic actions, no increase in peristaltic sounds and no colic, or other evidences that peristalsis was increased. The nausea and vomiting following the larger doses appeared to be of central origin, as in animals. In none of the patients were there any signs or symptoms which could be attributed to depression or paralysis of the sympathetic nervous system. Occasionally, the drug seemed to exert a mild hypnotic effect. Since the drug was administered systemically (intramuscularly) in all patients, the essentially negative results as to catharsis and related or accompanying effects could not be attributed to lack of absorption, as might be the case with oral administration. In our patients, the actions of the drug were not assisted or complicated by other drugs.

#### CONCLUSIONS

1 Apocodeine administered intramuscularly in doses varying from 12 to 97 mg to 19 normal patients, and to 3 patients with postoperative ileus, had no demonstrable effects on the motor functions of the intestine. Increase in peristalsis, as indicated by passage of stools, flatus, and increase in peristaltic sounds, was not demonstrated, and there was no cathartic action. There were indications of nausea and emesis in 27 per cent of the patients, occasionally also of narcosis. These essentially negative results on the alimentary tract under clinical conditions agree with recent negative pharmacologic results in animals.

2 Severe local reactions occurred after intramuscular injection of two apocodeine preparations, a green product and a specially prepared white product, thus indicating the irritant properties of the alkaloid, which according to other evidences, appears to be ill defined chemically.

3 According to the pharmacologic and clinical results obtained the clinical therapeutic use of apocodeine as available at present at least appears unwarranted

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## DETOXIFICATION WITH SPECIAL REFERENCE TO SODIUM RICINOLEATE I\*

T H RIDER PH D CINCINNATI

THE detoxification of bacterial toxins and other toxic material is a property which has been attributed by various authors to a great variety of compounds. Among these sodium ricinoleate is at present being used clinically for a variety of purposes. The present work has been undertaken to study the relationship between chemical structure and detoxifying power the first step of which has been to investigate the comparative detoxifying activities of sodium ricinoleate and the other products found in a crude castor oil soap. Comparatively little work has been done upon the detoxifying power of pure soaps so that no quantitative relationships have been established. In most cases the soap has been prepared from an oil and is referred to merely as a soap of that oil. Even where reference is made to purified soaps the purity is questionable. In the case of sodium ricinoleate a study of the various methods of purification which have been published prior to the method described by the writer raises doubt as to whether or not a truly pure sodium ricinoleate has ever before been prepared.

Davison<sup>3</sup> has studied the detoxifying action of many soaps and reports that their activity decreases in the following order

- 1 Castor Oil Soap
- 2 Coconut Oil Soap
- 3 Linseed Oil Soap
- 4 Cottonseed Oil Soap
- 5 Palm Oil Soap
- 6 Olive Oil Soap
- 7 Lard Soap
- 8 Peanut Oil Soap
- 9 Corn Oil Soap
- 10 Sesame Oil Soap
- 11 Mustard Oil Soap
- 12 Apricot Oil Soap

\* From the Research Laboratories of the Wm S. Merrill Company, Cincinnati, Ohio



The length of this list, every member of which Davison found to have some detoxifying power, makes necessary the conclusion that detoxification is a more or less general property of soaps, even though the actual detoxifying powers vary greatly.

Néls<sup>20, 21</sup> believes that sodium oleate behaves toward diphtheria toxin like a catalyst in the presence of which the toxin is definitely destroyed. Raubitschek and Russ<sup>24</sup> found sodium oleate to have an antitoxic effect on neurotoxins, and reported that this action is inhibited by serum, gelatin, and albumoses. Renaud<sup>26</sup> detoxified cobra venom by mixing it with solutions of sodium oleate, sodium palmitate or medicinal soap, the antigenic properties remaining undestroyed. Sédallian and Velluz<sup>20</sup> also noted the rather general occurrence of detoxifying ability among soaps and reported that the presence in the soap molecule of ethylene linkages and of an alcoholic hydroxyl group accentuated this property. Renaud<sup>25</sup> also called attention to the greater detoxifying power of sodium ricinoleate as compared to other soaps. Velluz<sup>31</sup> found that strychnine combined with various soaps to give atoxic compounds and that metal salts such as mercuric chloride were similarly rendered innocuous by soaps.<sup>32</sup> Vincent<sup>33, 34, 35, 36</sup> reported on the detoxifying powers of many soaps.

This paper does not attempt to give a critical analysis of the material on detoxification by soaps, since there is very general agreement that the property of rendering bacterial toxins atoxic is widespread, and good agreement also that sodium ricinoleate is the most active of the list in this respect.

The only claims against this generality of action were advanced by Laison<sup>14</sup> who states that soaps of oleic, stearic and palmitic acids have no detoxifying action whatever on bacterial toxins and that the presence of these soaps deprives sodium ricinoleate of its detoxifying power.

Sodium ricinoleate is prepared in crude form by the saponification of castor oil. A study of the preparation of a purified product must be based upon a knowledge of the composition of castor oil, since such a knowledge alone gives the necessary information as to the impurities which must be removed. A recent paper by Eibner and Munzing<sup>4</sup> assigns the following percentage composition to medicinal castor oil:

Ricinoleic acid	80
Oleic acid	9
Linolic acid	3
Dihydroxystearic and stearic acids	3
Glycerol and unsaponifiable matter	5

Little agreement is found in other estimates of composition. Krafft<sup>14</sup> discovered that when castor oil was chilled, 3 to 4 per cent of solid glycerides were deposited, in which he found stearic acid. Chonowsky<sup>2</sup> obtained similar results upon chilling an alcoholic solution of castor oil. Juillard<sup>12</sup> discovered about 1 per cent of dihydroxystearic acid. Hazuza and Giussnei<sup>10</sup> found that the oxidation of the liquid fatty acids gave two isomeric trihydroxystearic acids, and for this reason they believed that castor oil contained two isomeric ricinoleic acids. They found no oleic acid. Mangold<sup>18</sup> later showed that one unsaturated fatty acid may yield two isomeric hydroxy acids. Haller<sup>8</sup> found no isomeroleic acid and no oleic acid. Fahrion<sup>9</sup> found 2 to 3 per cent of linolic acid and 3 per cent of oleic acid. Lifschutz<sup>17</sup> has described a spectroscopic

method for the recognition of oleic acid in the presence of other fatty acids, and by application of this method to a study of the minimal concentrations of oleic acid and of castor oil which gave the characteristic spectrum, has estimated that the oleic acid content of castor oil is 0.6 to 0.8 per cent. In summary the composition as given by Eibner and Munzing is probably qualitatively correct, although there is reasonable doubt as to the quantitative accuracy.

Records of the preparation of castor oil soap go back to Rimmington<sup>28</sup> who in 1871 published a description of the saponification of castor oil. Sayre<sup>29</sup> saponified castor oil with sodium hydroxide and salted out the resulting soap with brine. Neison,<sup>10</sup> Heylmann,<sup>11</sup> Brown,<sup>1</sup> and Gordon<sup>6</sup> followed essentially the same procedure. Laison, Cantwell and Hartzell<sup>15</sup> saponified castor oil with an excess of alcoholic sodium hydroxide, neutralized the excess alkali with hydrochloric acid, filtered and threw the alcoholic soap solution into brine to remove the glycerol. The precipitated soap was washed with brine and dried.

The earliest attempt to prepare a purified sodium ricinoleate was that of Kiafft,<sup>14</sup> who in 1888, cooled the free fatty acids to 0° C and pressed the solid fatty acids at gradually rising temperatures, obtaining a solid acid melting near room temperature. Juillard<sup>12</sup> later showed that this method gave a product containing stearic acid and dihydroxystearic acids. Juillard attempted the purification of ricinoleic acid by filtering the crude acids to remove the insoluble solid fatty acids, then prepared the barium soaps of the liquid fatty acids and recrystallized them from alcohol. Decomposition of the barium soaps with hydrochloric acid gave a fatty acid product melting at 4-5° C. Haller<sup>8</sup> alcoholized castor oil with methyl alcohol and fractionated the resulting esters before resaponifying. Netter, Andre, Cesari and Cotoni<sup>22</sup> followed Haller's method and in addition purified the resulting fatty acids by the recrystallization of their barium salts from alcohol.

Kozłowski<sup>13</sup> and Hakonson<sup>9</sup> purified the barium soaps by repeated recrystallization from alcohol. Each fraction treated separately, a fraction of sodium soaps was obtained which had the theoretical iodine number for ricinoleic acid. This fact alone is not a complete criterion of purity, however, since a combined contamination with saturated fatty acids and the more unsaturated linolic acid might also give a product with the theoretical iodine number.

Fahnestock extracted castor oil and various ricinoleic acid derivatives with petroleum ether, and at a later date Panjutin and Rapoport<sup>23</sup> prepared a partially purified castor oil by extraction with benzine.

In checking the various methods outlined above for the preparation of a pure sodium ricinoleate, the writer found none of them to be satisfactory. The first problem was to remove the saturated stearic and dihydroxystearic acids. The chilling of an alcoholic solution of castor oil and the extraction of castor oil with petroleum ether produced some purification but were not investigated in great detail, since it is impossible to determine *a priori*, whether or not there exist in castor oil mixed glycerides which would retain a portion of the contaminating acids in the purified fraction. Chilling of the free fatty acids to a temperature which would precipitate all of the solid acids possible gave such a viscous liquid that it was impossible to filter it. The classical method for separating saturated and unsaturated fatty acids was then tried, namely

the ether extraction of the lead salts. When the ether soluble lead soaps were decomposed by hydrochloric acid, the free fatty acids obtained had a high iodine number, and it was believed that all of the saturated acids had been removed. When the sodium soap was prepared however, its aqueous solutions were found to cloud slightly on chilling. This cloud formation on chilling of a dilute soap solution was found by the writer to be a much more accurate test for the presence of saturated acids than a determination of the iodine number since the latter cannot be corrected for the unknown amount of linolic acid present.

The purification effected by recrystallization of the barium soaps from alcohol was studied next. A hot alcoholic solution of the mixed barium soaps from the fatty acids of castor oil was cooled gradually to room temperature when more than half of the barium salts had precipitated. The solution was then filtered and further chilled. The last fraction of barium soaps to precipitate was decomposed with acid and it was found that the resulting fatty acids not only had a lower iodine number than those obtained from the ether-extracted lead soaps, but that an aqueous solution of the corresponding sodium soaps clouded badly.

While it is not impossible that these methods of purification if frequently repeated might eventually succeed in removing all of the saturated fatty acids they are obviously not very satisfactory. While the pure lead soaps of a saturated acid may be insoluble in ether and the corresponding pure barium soap may be insoluble in cool alcohol, both products are soluble to an appreciable extent in the respective solvents containing large amounts of the corresponding salts of monoleic acid.

The methyl esters of the combined fatty acids from castor oil were prepared and fractionation attempted. Little success was had, however, since the distillation had to be carried out in vacuo and the boiling points of all of the pure methyl esters are within a few degrees of one another. The combined methyl esters distilled together over a very small temperature range, so that fractionation was practically impossible.

It was finally found that by dissolving the fatty acids in an equal volume of 95 per cent alcohol and chilling, the solid acids precipitated almost quantitatively and left a supernatant solution which could be easily filtered. A soap prepared from the fatty acids purified as described above gave a solution which remained clear even when frozen.

The solid fatty acids thus separated amounted to 2.6 per cent of the total. After various attempts it was found that the stearic acid could be separated from the dihydroxystearic acid by ether extraction. The ether soluble acids thus separated melted at 65-68° C., and thus were comparatively pure stearic acid (M. P. 69.3°). The ether insoluble material melted at 190-210° and contained few free acid groups. It is probable that this compound is a lactone of dihydroxystearic acid, since saponification with alcoholic potassium hydroxide and precipitation of the free acid with a mineral acid gave a compound melting at 141°, the correct melting point for dihydroxystearic acid. By separation of aliquots of the two acids it was found that dihydroxystearic was present to the amount of 1.68 per cent and stearic acid 0.7 per cent of the original castor oil used.

An analysis of the liquid fatty acids after complete removal of the solid fatty acids should yield information as to the relative amounts of ricinoleic, oleic, and linolic acids present. A determination of the acetyl number would give direct evidence as to the amount of ricinoleic acid, since no other hydroxylated acid would be present. The iodine number would give an estimate of the total unsaturation. The unsaturation attributable to ricinoleic acid could be calculated, since it would be in a definite ratio to the acetyl number. The relative amounts of linolic acid and oleic acid present could be then calculated from a knowledge of the unsaturation in the sample attributable to them and their respective unsaturations.

Grun<sup>7</sup> found that when ricinoleic acid was boiled with acetic anhydride, instead of the expected acetylated ricinoleic acid, a compound was obtained, in part, in which two molecules of ricinoleic acid and one of acetic were combined. For this reason, instead of carrying out the suggested analysis on the free fatty acids, the methyl esters were prepared, and an analysis of the same type was performed. The completion of this study as outlined, together with the completion of the experimental knowledge of the amounts of saturated acids and an estimation of the amounts of glycerol and unsaponifiable matter leads to the following percentage composition for castor oil.

Glycerol and unsaponifiable matter	5
Dihydroxystearic acid	2
Stearic acid	1
Ricinoleic acid	85
Linolic acid	6
Oleic acid	1

This cannot be considered an exact analysis, but the writer believes that it is as accurate as any other analysis available.

From this it would appear that the soap prepared from the fatty acids purified by removal of the saturated acids would be approximately 94 per cent pure sodium ricinoleate, together with 5 per cent sodium linoleate and 1 per cent sodium oleate. Linolic acid oxidizes in the air, and due to this the product which has been manipulated acquires a red-brown color. This color can only be removed by a complete removal of the linolic acid.

A complete purification of ricinoleic acid is possible only by a method capable of removing linolic and oleic acids in addition to the saturated acids. As mentioned above, among the unsaturated fatty acids ricinoleic is the only one which contains a hydroxyl group. While the various methyl esters distill together without separation it is possible to materially raise the boiling point of methyl ricinoleate by acetylation. Since the contaminating acids will be unaffected the methyl ricinoleate may be separated by fractional distillation *in vacuo*.

Such a preparation was carried out and the resulting methyl acetyl ricinoleate was proved to be absolutely pure since *both* iodine and acetyl numbers were identical with those calculated for the pure product.

From this material a chemically pure sodium ricinoleate was prepared, probably for the first time. The chemical details of this purification have been published by the writer.<sup>8</sup>

THE COMPARATIVE DETOXIFYING ACTIVITIES OF SODIUM RICINOLEATE  
OF VARYING DEGREES OF PURITY

This part of the work represents an attempt to determine the effect of concomitantly occurring impurities upon the detoxifying activity of sodium ricinoleate of various degrees of purity. The first sample considered was a sample of crude castor oil soap, which has undergone no purification. This soap has approximately the following percentage composition, based on dry weight:

Sodium ricinoleate	90
Sodium oleate	1
Sodium linoleate	6
Sodium stearate	1
Sodium dihydroxystearate	2

Laison<sup>16</sup> claimed that sodium oleate and sodium stearate have no detoxifying powers whatever, and that they detract from the detoxifying powers of sodium ricinoleate. Many other authors attribute detoxifying power to sodium oleate. The effects of sodium linoleate and sodium dihydroxystearate have not been reported.

A quantitative comparison of the detoxifying activity of the crude soap of the above composition with that of chemically pure sodium ricinoleate will tell the extent to which the contaminating acids effect detoxification. Examination of the detoxifying power of a partially purified soap will give further information on this subject. The soap prepared by removal of the solid fatty acids will have approximately the following percentage composition:

Sodium ricinoleate	93
Sodium oleate	1
Sodium linoleate	6

Thus a comparison of this partially purified soap with the crude soap will show the effect of the saturated stearic and dihydroxystearic acids, while its comparison with the chemically pure soap will show the effect of the unsaturated contaminating acids, oleic and linolic.

A study of the detoxifying powers of each of the contaminating acids themselves in a pure form will also add to knowledge of their effects. Chemically pure samples of the sodium salts of stearic, dihydroxystearic, oleic and linolic acids were prepared. Sodium stearate is almost totally insoluble in water so that there was not the opportunity of studying the detoxifying power of this pure soap in a manner comparable to the study on sodium ricinoleate.

Sodium dihydroxystearate is only slightly soluble in water at room temperature, but a supersaturated solution was prepared with a concentration of 0.15 per cent. This solution has no detoxifying power, at least of the order exhibited by sodium ricinoleate, since 50 M. L. D. of tetanus toxin dissolved in one cubic centimeter of such a solution killed guinea pigs as rapidly as the untreated toxin.

Pure sodium oleate and pure sodium linoleate solutions in concentrations of one per cent and one-half per cent did show a definite detoxifying activity.

Before discussing the quantitative results it is necessary to discuss the method of testing. The minimum lethal dose of the writer's supply of tetanus toxin was accepted as approximately one-tenth of a milligram for a guinea pig of 250 grams. One hundred times this amount was used for each detoxification.

test, dissolved in each case in one cubic centimeter of the fluid to be injected, each solution containing a definite concentration of the soap to be tested. This large dose of toxin was used in order to avoid errors due to the weighing of small amounts, and also to avoid so far as possible errors due to the varying activity of various portions of the dried toxin, which in all probability was not entirely homogeneous.

It is possible that the concentration of the soap is the determining factor in detoxification, not the absolute amount present. For this reason, the concentration of the toxin was kept constant in every case, and the concentration of the soap was altered in the various solutions prepared.

The results, in so far as the guinea pig is concerned may be of one of three types: (1) no protection, i. e., death will occur as rapidly as if no soap were present, (2) complete protection, i. e., the pig will live until death from some other cause intervenes, and (3) partial protection, i. e., the pig will not die as rapidly as if no soap had been mixed with the toxin, but will die from tetanus after a somewhat longer interval.

Due to the unknown length of time which a pig would have to be watched to report that the dose had proven absolutely atoxic, the simplest comparative tests are obtained by determining the prolongation of life, compared to a control animal which received straight toxin, of an animal receiving the toxin with a definite concentration of soap. The results found in this quantitative study of the detoxifying powers of the various soaps are summarized in the following tables. The length of the line of crosses in each case represents the average length of life of guinea pigs which had been injected with 100 M. L. D. of tetanus toxin mixed with the designated concentration of the soaps. The final cross signifies the time of death. Each case shown represents the average results of experiments on at least three guinea pigs, since in no animal work can the results be considered typical unless they are consistent in the cases of at least three different individuals.

A series in which the soap concentrations were 0.1 per cent gave partial protection to a very slight degree. In this series there is a slight indication that the C. P. sodium ricinoleate is a more powerful detoxifying agent than the less pure samples, but the differences are very slight. The results are represented graphically as described, each cross representing a period of two hours.

Sodium Ricinoleate 0.1%	
Soap Used	Period of Survival
None—control	XXXXXXXX
Crude	XXXXXXXX
Purified	XXXXXXXXXX
C. P.	XXXXXXXXXX

The same general results are found in the series of pigs injected with the toxin modified by solution in 0.2 per cent soap solutions. This group may be represented graphically as follows, omitting the first part of each line.

Sodium Ricinoleate 0.2%	
Soap Used	Period of Survival
None—control	X
Crude	XXXXXXXXXXXXXXXXXXXX
Purified	XXXXXXXXXXXXXXXXXXXX
C. P.	XXXXXXXXXXXXXXXXXXXX

Here the differences in the results with the soaps of the three degrees of purity are more marked. It must be remembered, also that the crude sample only contains nine tenths of the sodium ricinoleate in the corresponding C P sample and that if the soaps composing the other tenth were totally inactive we should expect only nine-tenths of the activity of the pure soap to be apparent in the crude soap. Laison's claims that such soaps as sodium oleate and sodium stearate materially detract from the detoxifying powers of sodium ricinoleate cannot be valid for the concentration in which they are found in the crude castor oil soap, since there is no marked increase in activity when they are removed.

In experiments with the soaps present in higher concentration, the same results are borne out. Pigs receiving 100 M L D tetanus toxin mixed with 0.25 per cent of the C P soap survived on the average for 147 hours while those with the same concentration of crude soap died in 102 hours.

Where 0.3 per cent soap was used, the pigs in the series where the crude soap was used all showed signs of tetanus and two thirds of them died between the seventh and eighth day. In the corresponding experiments where the C P soap was used the animals showed no signs of tetanus and all were alive after eight days. At the end of this time several animals protected by still larger concentrations of soap had died without tetanic symptoms so that the results from here on cannot be considered accurate.

It has already been stated that sodium dihydroxystearate and sodium stearate do not compare with sodium ricinoleate as detoxifying agents. Sodium oleate and sodium linoleate in 1 per cent solution both give perfect protection to pigs. The following table will give an idea of the comparative quantitative detoxifying powers.

SOAP	CONCENTRATION	DEATH AFTER
Controls		16 hr
Sodium Oleate	1.0%	
	0.5%	35 hr
Sodium Linoleate	1.0%	
	0.5%	80 hr
	0.3%	41 hr
	0.2%	24 hr
Crude Sodium Ricinoleate	0.5%	
	0.3%	7 days
	0.2%	54 hr

#### SUMMARY

Crude castor oil soap consists of sodium ricinoleate contaminated with sodium oleate, sodium stearate, sodium dihydroxystearate and sodium linoleate. The two saturated compounds, i. e., the stearate and dihydroxystearate do not have detoxifying powers, at least not of the degree found in sodium ricinoleate. They are present in only small quantities, and their removal gives a product of definitely but slightly increased detoxifying powers. Sodium oleate and sodium linoleate both have definite detoxifying powers which are, however, considerably less than those of sodium ricinoleate. Additional purification by the removal of these soaps again gives a definite but slight increase in activity. There are, however, manifold other advantages to purification which need not be discussed at this time.

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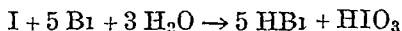
## BLOOD IODINE STUDIES

### I THE QUANTITATIVE DETERMINATION OF THE IODINE CONTENT OF BLOOD\*

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THAT there exists an interrelationship between the thyroid gland and iodine has long been recognized. The discovery of considerable amounts of iodine within the gland, by Baumann, is but one of the important steps in the development of this conception. It is now well established that iodine is a constant constituent of the blood. Recent investigation is attempting to define more clearly this interrelationship in thyroid disease, particularly in regard to the iodine content of the blood, of the thyroid gland, and to a certain extent of the other tissues. One difficulty in the progress of this study has been the development of adequate micromethods for determining the small amounts of iodine normally present within the blood and tissues. An answer to this has been furnished within the past decade, particularly in the work of von Fellenberg. Much remains to be learned regarding the interrelationship between the thyroid, iodine metabolism, and goiter. As a consequence, it has seemed worth while to develop available methods for the determination of the small amounts of blood iodine and to apply them in more extensive metabolic studies. It is true to state that the application of the newer methods is of value in the development of knowledge in such a field. We wish to present at this time the method with which Davis experimented for five months and which subsequently proved, in his hands, successful. Later we shall present results which we have obtained in applying this method to the study of the blood in the various diseases of the thyroid gland.

The principles upon which the method we have used is based were developed by Winkler<sup>1</sup> who made use of the fact, previously discovered by him,<sup>2</sup> that an iodide may readily be oxidized to an iodate \*\*. In our work we have used biomine for this oxidation, according to the formula



The iodate thus formed readily reacts with an excess of hydriodic acid (KI in

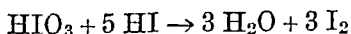
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\*\*Owing to the significance of this contribution commonly known as the Winkler principle in the development of modern micromethods for the determination of iodine it is of interest that A. and F. Dupré<sup>3</sup> working in Heidelberg under Bunsen had a similar method in mind. They showed when iodine is gradually liberated from iodides by free chlorine water that the extent to which the iodine may be shaken out by carbon disulphide or chloroform depends upon the amount of chlorine water added, but that if one adds a sufficient excess of chlorine water none of the iodine remains free to be dissolved in the carbon disulphide or chloroform. They considered that the inorganic iodide was converted into free iodine then ICl and finally ICl<sub>3</sub> as the amount of chlorine was increased. This ICl<sub>3</sub> when later treated with excess KI then gave 6 parts of iodine for each part of ICl<sub>3</sub>. Thus their ratio is the same as in the present method. Their interpretation of the reaction however is probably incorrect since the formation of iodate is now well established. We are indebted to Prof. F. C. Koch for this information and for other valuable suggestions.

acid solution), liberating six parts of iodine for each part present in the original substance to be analyzed, according to the formula



Thus is developed a six-fold greater titration value than that which would be given by the iodine originally present in the sample. It can readily be seen that this is of major importance in the analysis of material, such as blood, in which the iodine content is so low that it is given in terms of *gamma*. One gamma equals 0.001 mg. In this region the blood iodine of nongoutous clinic and hospital patients averages 12 gamma per cent.

Von Fellenberg,<sup>3</sup> Leitch and Henderson,<sup>4</sup> McClendon<sup>10</sup> and others have utilized this principle, working it into methods adapted for use in the analysis of material containing much organic matter and but little iodine. To von Fellenberg should go most of the credit for developing the method whereby the organic matter is eliminated without loss of iodine. Von Fellenberg's method has been widely utilized by Lunde and his associates and by Sturm.

The material to be analyzed, in this instance blood, is first ashed by heating with KOH, after which the iodine is extracted as KI. The extract is acidified and the resulting HI is oxidized to HIO<sub>3</sub> by boiling with bromine water. The HIO<sub>3</sub> is then allowed to react with an excess of KI (still in acid solution) and the iodine set free is titrated against N/500 sodium thiosulphate using starch solution as an indicator.

This titrimetric method, as employed by von Fellenberg,<sup>3</sup> Leitch and Henderson,<sup>4</sup> Veil and Sturm,<sup>5</sup> Lunde and Closs,<sup>6</sup> and others has been fully described. We are repeating it here as we have utilized it in order to give the benefit of our particular experience with the method and to point out certain of the numerous pitfalls which will be mentioned later under discussion of the method.

#### REAGENTS

*Potassium hydroxide—40 per cent*—This reagent is made by dissolving 400 grams of KOH, "Kahlbaum," in 600 c.c. of distilled water and diluting to 1000 c.c.

*Potassium nitrate—15 per cent*—One hundred and fifty grams of KNO<sub>3</sub>, "Kahlbaum," is dissolved in distilled water and diluted to 1000 c.c.

*Ethyl alcohol—90 per cent*—Redistilled.

*Sulphuric acid—2N*—Add 56 c.c. of concentrated H<sub>2</sub>SO<sub>4</sub> to 100 c.c. distilled water. This solution will keep indefinitely.

*Potassium iodide—10 per cent*—Dissolve 5 grams in 50 c.c. of water. Make up fresh each time used. This solution need only be approximate.

*Bromine water*—A saturated solution, made by shaking up 2 c.c. of bromine in 100 c.c. of distilled water. Owing to the possibility of some bromic acid formation, this should be prepared fresh every few days and kept in the dark.

*Methyl orange*—0.05 per cent solution.

*Potassium bismodate—N/10*—This, the real standard used, is made by dissolving 3.2496 grams of potassium bismodate (very accurately weighed) in water and diluting to one liter. Shaffer and Hartmann<sup>11</sup> give excellent directions for preparing a very high grade potassium bismodate.

*Sodium thiosulphate—N/10*—Dissolve 25 grams of the pure crystalline salt

in recently boiled distilled water. Dilute to 1000 cc with recently boiled distilled water. Standardize this as follows. Measure off 25 cc of the N/10 potassium bismutate into a 250 cc Erlenmeyer flask. Add 100 cc distilled water, 1 gram KI and 5 cc of 10 per cent HCl. Titrate at once to a light yellow color with the sodium thiosulphate. Add 1 cc of the 1 per cent starch solution and titrate to a colorless end-point. Due to the large amounts of reagents used this end-point is sharp and offers no difficulties. The thiosulphate solution is then diluted until it is within 0.2 per cent of N/10. Further accuracy is not needed. Acidity and microorganisms cause decomposition of the sodium thiosulphate. As a consequence it should be protected from atmospheric carbon dioxide and contamination and kept in the dark, or it should be rendered slightly alkaline by adding sodium carbonate as recommended by Mayr and Kerschbaum.<sup>14</sup> N/500 sodium thiosulphate is made fresh from this stock solution each time as needed, by accurately diluting 2 cc to 100 cc.

*Starch solution*—1 per cent—Made by mixing 1 gram soluble starch with 100 cc of distilled water and boiling 1 minute.

#### PURITY OF REAGENTS

Kahlbaum's reagent grades of KOH and KNO<sub>3</sub> have been repeatedly tested for iodine content. Ten times the amount of these reagents used in the determination will give no trace of color with the starch solution when run as a "blank." The alcohol should be similarly tested and if found to be contaminated by iodine should be redistilled from solid KOH.

To test the H<sub>2</sub>SO<sub>4</sub> and bromine water, titrate 5 cc of the 2N acid with KOH using methyl orange as an indicator. Bring the solution back to the acid side of the indicator with 1 drop of the acid. Add 5 cc of bromine water and boil until the volume of the solution is about 2 cc. Add 5 drops of 10 per cent KI and 5 drops of 1 per cent starch solution. There should be no trace of color, as a blue color at this point would indicate the presence of iodine in the bromine. Iodine is a common impurity in bromine and the bromine should be tested carefully.

Ordinary C. P. sodium thiosulphate and potassium iodide are sufficiently pure.

#### PROCEDURE

In drawing blood from the patient great care must be taken to avoid contamination. A chemically clean but not necessarily sterile syringe is used. The blood is taken from one of the cubital veins after carefully cleansing the skin with pure alcohol. *No iodine is used and there should be no iodine on the clean-up tray.* At least 22 cc of blood should be drawn and divided immediately between two one ounce bottles, each containing 5 mg of sodium oxalate or sodium citrate. Either oxalate or citrate may be used. The bottles should be shaken thoroughly by rapid rotation.

By means of an outvolume pipette, measure accurately 10 cc of whole blood into a 50 cc nickel crucible. Add 25 cc of 40 per cent KOH solution and stir with a fine-pointed glass rod. Rub the adherent mixture off the tip of the rod against the side of the crucible. It is impractical to rinse off the rod. Put the crucible containing the mixture into an oven kept at 120° C and allow it to remain there for at least twelve hours. This dries the blood so

that it can be heated to a char without excessive bubbling. *The oven used in this and succeeding steps should never be used for any other purpose than for iodine determinations.* After drying the blood and KOH mixture is heated to a char. This heating is done by placing the nickel crucible in a large iron crucible about 8 cm. in diameter. In the bottom of the iron crucible is a layer of sand about 5 mm. deep. The iron crucible is then heated over a Bunsen burner in a hood. In this and in succeeding heatings the nickel crucible should never be heated to a glow. When the mixture has become a dry char the two crucibles are transferred to a Chaddock burner and the heating continued. At ten-minute intervals the nickel crucible is taken out and allowed to cool. The contents are moistened with 15 per cent  $\text{KNO}_3$  solution and the nickel crucible is returned to the iron one. This procedure is continued until all the carbon particles are oxidized and the content is a fused reddish mass.

Distilled water is then added to the ash in 3 c.c. portions. Each portion, after standing a few minutes, is filtered through a No. 40 Whatman filter paper into a second nickel crucible. After the ash has been all dissolved, the crucible is rinsed with several 3 c.c. portions of water and the rinsings are also poured through the filter. Rinse the filter paper once and discard it, since repeated determinations have failed to detect the presence of iodine. The crucible containing the filtrate is now transferred to the  $120^\circ$  oven and evaporated to dryness on a grid in the upper part of the oven.

Enough distilled water is added to the residue to dissolve it on heating. This requires 1 to 1.5 c.c. Return to the oven and evaporate until the solution begins to solidify. Add 3 c.c. of the 90 per cent alcohol while still hot. Cool and rub the KOH mixture to a paste. This is best done with a nickel or iron rod about 3 mm. in diameter with a chisel-shaped end. *It is extremely important that there should be just the right amount of water left in the KOH mixture when the alcohol is added.* Recognition of this point is a matter of experience. The best criterion lies in the amount of 2N sulphuric acid required to titrate the alcoholic extract. This should be between 10 and 20 drops. When more acid must be used, the  $\text{K}_2\text{SO}_4$  interferes with the final titration, when less is used, the solution is not buffered sufficiently and the acidity developed is so high that the KI added is oxidized by atmospheric oxygen.

The alcohol is decanted off and filtered through a No. 40 Whatman filter into a 50 c.c. Erlenmeyer flask. The paste is then extracted by three more 3 c.c. portions of 90 per cent alcohol. Each of these portions should be allowed to drain through the filter before the succeeding one is decanted into the filter. After the last extraction, the paste is transferred to the filter and allowed to drain for thirty minutes. The funnel is rinsed once after the filter has been removed. The Erlenmeyer flasks are then transferred to the oven and the filtrate evaporated to dryness.

The flasks containing the evaporated filtrate are removed from the oven, cooled, and the film of salts is dissolved in 3 to 5 c.c. of distilled water. Two drops of the 0.05 per cent methyl orange solution are added and the solution is titrated to the methyl orange end-point with 2N  $\text{H}_2\text{SO}_4$ , one drop of acid being added in excess. This titration is a delicate one. The red color of the methyl orange on reaching the acid side of the end-point is bleached almost

immediately if much  $\text{HIO}_3$  is present so the end-point must be approached cautiously. Bromine water is added drop by drop until the solution is distinctly yellow. Two small pieces of pumice stone are dropped into each flask and the solutions are boiled until the excess bromine is driven off and the volume of the solutions is from 0.5 to 1 c.c. The flasks are cooled and from 2 to 5 drops of 10 per cent KI solution and 2 drops of starch solution are added to each flask. First add 2 drops of KI, if the solution turns yellow, add 3 more.

The solutions are now titrated with freshly prepared N/500 sodium thiosulphate solution. For this titration is used a 0.2 c.c. microburette, graduated in thousandths of a cubic centimeter, and with an attached reservoir. The pipette tip of this burette is drawn out to a fine point. With this pipette three cubic millimeters (0.003 c.c.) can be accurately delivered by means of the burette stopcock. The minute droplet so delivered is added by touching the tip to the flask containing the solution which is being titrated. As the end-point is approached, the thiosulphate is added in 0.003 c.c. increments. When the point is reached where it is doubtful as to whether any color remains, we usually add a full drop. Then, if there is any further color change, we add 0.002 to 0.005 c.c. to the titration readings according to the amount of color change.

#### CALCULATION

The calculation is based on the formula

Thiosulphate used  $\times \frac{\text{iodine equivalent of thiosulphate}}{6}$  N/500 iodine contains  $\frac{127}{500}$  grams per liter. Hence, 1 c.c. of N/500 sodium thiosulphate is equivalent to  $\frac{127}{500 \times 1000}$  or 25.4 gamma of iodine. Consequently  $\frac{25.4}{6}$  or 4.23 gamma is the iodine equivalent, in the original sample, of each cubic centimeter of thiosulphate. Since 10 c.c. blood samples are used the gamma per cent of the blood iodine is 42.3 times the number of cubic centimeters of sodium thiosulphate used in the final titration.

#### DISCUSSION

This method for the quantitative determination of the blood iodine is by no means an easy one. We feel that its success in the ordinary clinical laboratory would be doubtful. It requires experience and practice to obtain satisfactory results. It is best carried out in a laboratory used solely for that purpose and one in which no tincture of iodine or other volatile form of iodine is ever allowed to be present. Such reagents used in the procedure as contain iodine should be handled with extreme care to prevent possible iodine contamination of the blood samples.

After five months of thorough experimentation, this method was applied to samples of starch solution to which had been added known quantities of iodine. Davis was able to differentiate definitely between samples which contained 0.8 gamma, 8 gamma per cent, and those which contained 1.0 gamma, 10 gamma per cent. After working with about 500 samples during the past eighteen months, Davis can make duplicate determinations on blood samples containing 0.8 to 1.2 gamma with a variation between duplicates of about 0.05 gamma, or an error of from 6 to 8 per cent. In succeeding papers we shall

report studies on the blood iodine in normal individuals and in patients with diseases of the thyroid gland

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## THE EFFECT OF SODIUM THIOCYANATE ON THE ACTION OF ANESTHETIC AND NARCOTIC DRUGS\*

THEODORE M. BURKHOLDER, B S, CHICAGO, ILL

**W**ILDER D Bancroft and J E Rutzler, Jr<sup>1</sup> have presented several experiments in vivo which they believe confirm Claude Bernard's theory of the phenomenon of anesthesia, which is Anesthesia results when the colloids of the sensory nerve cells are reversibly coagulated (the coagulating agent is the anesthetic) and if the anesthetic is displaced by a substance that causes weaker flocculation, the biocolloids are peptized by the electrolytes within the cells and there is a return of irritability and consciousness These two workers chose sodium thiocyanate as the peptizing agent in most of their experiments They believe that the sensory nerve colloids are albumen-like and should adsorb the thiocyanate ion strongly and be easily peptized by the cell electrolytes

In their paper they reported but one experiment each with the following anesthetic drugs ether, sodium amytal, morphine, alcohol, and sodium nembutal, and also one experiment each with anaphylaxis, histamine, and strychnine poisoning

The experimental technic used in their report does not seem adequate for

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conclusive results In both the ether and amytal experiments the rabbits were chosen so young (weight 600 gm) that they could not be deemed normal mature animals

In the experiments in which the sodium thiocyanate was used as an antagonist for ether, the control animal, which received ether alone a few hours later underwent the sodium thiocyanate and ether combination Certainly the second time this rabbit could not be considered normal and probably required much less ether to depress it so that its recovery was much more rapid

Two young rabbits were used in the experiment with sodium thiocyanate and sodium amytal Bancroft and Rutzler stated in their publication that the hind legs of the experimental animal were not normal during the recovery period while the control animal was normal Had they performed more than one such experiment they would have noticed that every animal which receives the full dose of sodium thiocyanate shows this peculiar inability to control the hind legs, and as the dose is increased, the paralytic effect increases until all the legs are paralyzed and death often follows within twenty-four hours We found this paralytic effect in our animals which received sodium thiocyanate and morphine in the same dosages as employed by Bancroft and Rutzler Upon reducing the dosage of sodium thiocyanate only the effect of the morphine was evidenced

The fact that only one experiment with each anesthetic was performed by these workers limits the value of their results In experiments where two animals were used, the one receiving the thiocyanate might easily have been a rabbit which threw off the anesthetic more rapidly than the control since there is a great variation in reaction to anesthetics by different animals of the same species as well as the same animal However, in each of their experiments the results observed seemed to confirm their theory that sodium thiocyanate would increase the rate of recovery from anesthesia produced by these agents as well as from anaphylactic shock and strychnine poisoning They conclude that

1 "Sodium thiocyanate relieves to a greater or lesser extent the effect of these drugs"

2 The action of the sodium thiocyanate is due to the thiocyanate ion which peptizes the colloids that are coagulated by the above mentioned drugs

3 "Sodium thiocyanate apparently peptizes the colloids of the respiratory center" This theory is based on the fact that with amytal the respiratory recovery was 130 per cent faster in the experimental animal than in the control animal

4 "The use of too much sodium thiocyanate in the presence of a strong narcotic may cause sodium poisoning"

5 Sodium thiocyanate may relieve drug addiction by the peptizing of the nerve colloids of the brain

6 The thiocyanate ion acts upon the protein of the central nervous system, including the brain, spinal cord and sympathetic nerves

7 "No evidence has been found or adducted that the lipoids play a dominant rôle in the phenomena studied"

8 "Claude Bernard's theory of anesthesia as developed by Bancroft and Richter has been found to be inescapable"

In subsequent papers Bancroft and his associates<sup>1</sup> have discussed the application of this theory to the treatment of insanity, alcoholism and drug addiction

In this laboratory an attempt has been made to follow with adequate controls the exact experiments described by Bancroft and Rutzler, using sodium thiocyanate with ether, morphine, and sodium amytal, in order to determine

whether such results as they observed in a very limited number of experiments are constant phenomena justifying important conclusions

## EXPERIMENTAL STUDY

### I ETHER

Experiments using ether and sodium thiocyanate were performed on rabbits. The type experiment consisted essentially of placing a rabbit upon a rabbit board with its back down and securely tied. A weighed amount of sterile gauze was placed in a small ether mask and 10 cc of Squibb's pure ether was poured upon it. The mask was placed firmly over the rabbit's nose. The animal usually struggled violently at first, due to displeasure produced by the odor of the ether. Later they struggled because they were entering the excitement stage. The administration of the anesthetic was continued for ten seconds after the lid reflex had disappeared. This is a very deep state of anesthesia. Then the ether mask was removed and the animal freed. Records were taken of the time of the return of the lid reflex, of the time when the animal sat up, and finally the complete return to normal. Return to normal was judged by the ability of the animal to hop without staggering.

The same animal was then allowed several hours or even a day to rest. The experiment was then repeated exactly with the exception that during the last minute, while the ether was being given, sodium thiocyanate solution was injected into the marginal vein of the ear. (The amount of sodium thiocyanate was 0.00015 gm per gram of body weight which was the same as used in the Bancroft-Rutzler experiment. A 10 per cent solution was used.) The records were then compared with those of the controls.

On July 15 three healthy, young, snuffle-free rabbits were selected and a control run on each as described above. The results are as follows:

Rabbit A 206—Weight 1007 gm Temp of room 82° F (1) Lost lid reflex in three minutes, twelve seconds (2) Removed ether mask in three minutes and twenty two seconds (3) Lid reflex returned in three minutes and forty five seconds (4) Considered normal in twenty six minutes and forty three seconds

In this case twenty three seconds elapsed between the removal of the mask and the return of the lid reflex. The animal was normal in twenty three minutes and twenty one seconds.

Rabbit A 164—Weight 1545 gm (1) Lost lid reflex in two minutes and fifty seconds (2) Removed ether mask in three minutes (3) Return of lid reflex in three minutes and twenty three seconds (4) Considered normal in twenty four minutes

Lid reflex returned in twenty three seconds and the animal was considered normal in twenty one minutes after the ether mask was removed.

Rabbit A 173—Weight 1440 gm (1) Lost lid reflex in two minutes and fifty three seconds (2) Removed mask in three minutes and three seconds (3) Returned lid reflex in three minutes and twenty seven seconds (4) Considered normal in twenty five minutes

Lid reflex returned in twenty four seconds and the animal was considered normal in twenty one minutes and fifty seven seconds after the ether mask was removed.

These controls show that the time of the return of the lid reflex was nearly constant. The time was twenty three, twenty three, and twenty four seconds respectively. The time for the return to normal varied but slightly, as follows:

Rabbit A 206, twenty three minutes and twenty one seconds, A 164, twenty one minutes, A 173, twenty one minutes and fifty seven seconds.

This shows but little individual variation.

Six days later (this seemed to be time enough for the rabbits to be considered normal) July 21, these same three rabbits were used in the identical manner with ether plus sodium thio



cyanate using a 10 per cent solution and giving 0.00015 gm per gram body weight. This thiocyanate solution was administered intravenously into the ear vein during the last minute of anesthesia. The results are as follows:

Rabbit A 206—Weight 1007 gm received 1.5 cc of 10 per cent solution of sodium thiocyanate. (1) Lost lid reflex in four minutes and thirty seconds. (2) Removed ether mask in four minutes and forty seconds. (3) Lid reflex returned in four minutes and fifty-seven seconds. (4) Considered normal in twelve minutes and five seconds.

The lid reflex in this case returned in seventeen seconds and the return to normal was in seven minutes and twenty-five seconds. This animal died two days after the experiment.

Rabbit A 164—Weight 1545 gm received 2.3 cc of 10 per cent solution of sodium thiocyanate. (1) Lost lid reflex in three minutes and eighteen seconds. (2) Removed ether mask in three minutes and twenty-eight seconds. (3) Return of lid reflex in three minutes and thirty-nine seconds. (4) Considered normal in eleven minutes and five seconds.

The lid reflex returned in eleven seconds and the rabbit was normal in seven minutes and thirty-seven seconds.

Rabbit A 173—Weight 1440 gm received 2.16 cc of 10 per cent solution of sodium thiocyanate. (1) Lost lid reflex in six minutes and eighteen seconds. (2) Removed ether mask in six minutes and twenty-eight seconds. (3) Return of lid reflex in six minutes and forty-five seconds. (4) Considered normal in fourteen minutes and two seconds.

The lid reflex returned in seventeen seconds and the rabbit was normal in seven minutes and thirty-four seconds after the ether mask was removed. It died three days later.

The following chart summarizes

Return of lid reflex		Return to normal
Rabbit	(After mask is removed) <i>Ether</i>	
A 206	23 sec	23 min 21 sec
A 164	23 sec	21 min 0 sec
A 173	24 sec	21 min 57 sec
<i>Ether plus Sodium Thiocyanate</i>		
A 206	17 sec	7 min 25 sec
A 164	11 sec	7 min 37 sec
A 173	17 sec	7 min 34 sec

This set of three rabbits, in which the control was run first, certainly gave results which point toward a more rapid return of the lid reflex and the return to normal with the use of sodium thiocyanate than without it. The results show little variation.

A reversal of the order of the experiment was then made, running the experiment first, followed by the controls. The same procedure was followed, using three new rabbits. One day was allowed for recovery between the experiment and the control.

The following chart summarizes

Return of lid reflex		Return to normal
Rabbit	(After ether mask is removed) <i>Ether plus Sodium Thiocyanate—July 20</i>	
A 119	35 sec	23 min 50 sec
A 123*	25 sec	10 min 8 sec
A 116*	32 sec	15 min 44 sec
<i>Ether—July 21</i>		
A 119	22 sec	17 min 20 sec
A 123	22 sec	17 min 0 sec
A 116	20 sec	19 min 44 sec

\*Rabbits A 123 and A 116 died four and five days later respectively.

In this set of experiments the reversal of the order gave results which are contradictory to those of the first set as far as the return of the lid reflex is concerned. The return to normal with the use of thiocyanate in numbers A 123 and A 116 was more rapid than with the controls, as Bancroft and Rutzler stated, but much slower with A 119, which is just opposite to their theory. The recovery of A 123 with thiocyanate was only about half as rapid as in the preceding group, while all the lid reflexes required a much longer time in this group. We have, then, a decided variation in rate of recovery between the two groups. Bancroft and Rutzler did not report a reversal of the order of the experiment and therefore were not aware of the fact that the animals do not show the same rate of recovery when the experiment precedes the control as they do when it follows the control.

It was noticed soon after the injections of the sodium thiocyanate solution that the intestines were in exceedingly active motion. This was not noticed in the control animals.

Of the six animals used in these two groups of experiments four developed intestinal disturbances with diarrhea and died within two to five days after the injection of the thiocyanate. During this stage they refused food, lost weight rapidly, became quite weak and died with slight convulsive jerks of their legs and body and marked extension of the hind legs. Autopsy revealed no recognizable lesions or changes in the organs. These toxic symptoms were not observed by Bancroft and Rutzler.

An experiment was then run upon one rabbit to determine if there were any individual variations with the use of ether alone which might account for the fact that one rabbit contradicted their results.

Rabbit A 165 was run on July 13 with ether only. (1) Lost lid reflex in ten minutes and eight seconds. (2) Removed ether mask ten minutes and twenty eight seconds. (3) Return of lid reflex twelve minutes and eight seconds. (4) Considered normal in twenty five minutes and ten seconds.

Two days later, July 15, this same rabbit was run with ether again. (1) Lost lid reflex in three minutes and twenty five seconds. (2) Removed ether mask in three minutes and forty five seconds. (3) Reflex of lid returned in four minutes and twenty seconds. (4) Considered normal in twenty two minutes and fifteen seconds.

In the first case the lid reflex returned in one minute and forty seconds after stopping the anesthetic and in the second case thirty-five seconds. It took much longer in the first experiment for the rabbit to lose consciousness. Perhaps it was becoming accustomed to ether by the time of the second experiment. It will be noticed in the first experiment that the return to normal was fourteen minutes and forty-two seconds while in the later experiment it required eighteen minutes and thirty seconds, although the return of the lid reflex was much faster than in the first experiment. Evidently there is some individual factor to be considered in these experiments and the variations obtained before cannot be attributed to thiocyanate alone.

These results called for an experiment with a fresh rabbit using ether and sodium thiocyanate together and repeating in a few days, to see if there is any individual variation with thiocyanate.

Rabbit A 168, weighing 1640 gm was selected July 29. It received the usual

10 cc of ether, followed by 2.46 cc of 10 per cent solution of sodium thiocyanate in the marginal vein of the ear during the last minute of anesthesia.

The lid reflex returned in twenty-six seconds and the animal was normal in nine minutes and twenty-one seconds. Two days later the same experiment was run on the same rabbit. This time the lid reflex returned in twenty-three seconds and the animal was normal in five minutes and one second. This shows a marked increase in the rate of recovery during the second experiment, although the same technique and the same dosages were used. If the thiocyanate had not been used in the first of these tests, we could easily have believed that the thiocyanate had caused the more rapid recovery in the second test. This indicates that the increased rate of recovery with the use of thiocyanate in the earlier experiments cannot be definitely attributed to the use of that drug.

Rabbits A 73 and A 166 were run in like manner at the same time, using ether and 10 per cent solution of sodium thiocyanate.

Rabbit A 73 had a return of lid reflex in twenty seconds and was normal in five minutes and forty-two seconds. Two days later in the second run the lid reflex returned in eleven seconds and the animal was normal in five minutes and forty-six seconds. Here the return to normal was almost an exact check.

Rabbit A 166 had a return of the lid reflex in twenty seconds and was normal in five minutes and ten seconds and in the second run two days later the lid reflex returned in twenty-seven seconds and the animal was normal in four minutes and twelve seconds. We can conclude that rabbits A 168 and A 167 show individual variations while A 73 is quite constant.

This is another experiment which shows that rabbits do not always react in the same way to sodium thiocyanate when it is used as an antagonist for anesthetic drugs.

Rabbit A 166 died six days later and rabbit A 168 died ten days later with no gross nervous lesions, but both did show loss of weight.

At this point it became evident that conclusive results could not be obtained by this procedure since marked deviations continued to occur in experiments that were identically the same. However, experimentation was continued with the intention of giving the problem a fair trial.

Two fresh rabbits were subjected to the usual ether-sodium thiocyanate experiment and followed eleven days later by the control. Rabbits A 143, weight 1650 gm and A 95, weight 1730 gm received respectively 2.47 cc and 2.59 cc of 10 per cent sodium thiocyanate solution during the last minute of anesthesia. Rabbit A 145 returned to normal in eight minutes and fifty-seven seconds and A 95 was normal in five minutes and thirty-nine seconds, although both experiments were conducted in identically the same manner. Eleven days later these two rabbits underwent the control experiment using ether alone. Rabbit A 143 returned to normal in six minutes, or two and fifty-seven seconds quicker with ether alone than with thiocyanate.

However Rabbit A 95 required ten minutes and fifty-four seconds to return to normal with ether or nearly twice as long as with thiocyanate. This experiment is more in accord with the results obtained by Bancroft and Rutzler and might have led them to believe that they had discovered an antagonist for anes-

thesia Nevertheless this increased rate of recovery with thiocyanate may not be due to the effect of this drug but simply another example of individual variation such as has been seen before

The fact that this one experiment was similar to the reports of the Cornell workers led to a group experiment on four more rabbits These rabbits were selected so that they were of the same sex and very near the same weight Two were run in the usual way as controls with ether alone and two received the ether-thiocyanate treatment

The chart shows the result of these four experiments

Morning Ether			
Rabbit	Weight	Return of lid reflex	Normal
A 185	1720 gm	21 seconds	14 min 42 sec
A 187	1880 gm	15 seconds	17 min 17 sec
Afternoon Ether and Sodium Thiocyanate			
A 185	1720 gm	28 seconds	16 min 22 sec
A 187	1880 gm	59 seconds	10 min 48 sec
Morning Ether and Sodium Thiocyanate			
A 31	1880 gm	14 seconds	10 min 17 sec
A 39	1770 gm	59 seconds	10 min 2 sec
Afternoon Ether			
A 31	1880 gm	21 seconds	11 min 0 sec
A 39	1770 gm	38 seconds	8 min 27 sec

Although this experiment was run the same day, the experiments were reversed so that the two rabbits receiving the ether alone in the morning received ether and sodium thiocyanate in the afternoon, and those that received sodium thiocyanate in the morning received ether in the afternoon The results showed that rabbit A 187 recovered more quickly to normal under the influence of sodium thiocyanate than when it had ether only However the rabbits A 185 and A 39 took longer to recover with the use of sodium thiocyanate Rabbit A 31 returned to normal in almost the same time with or without thiocyanate

Seven out of seventeen rabbits in these experiments which received the regular dose of sodium thiocyanate died within two to ten days showing only weakness, loss of weight and diarrhea Autopsy revealed no gross lesions

It was thought that the sodium ion might have some effect on these results, and so three fresh rabbits were selected and anesthetized with ether as before, and an equimolar solution of sodium chloride as that of the sodium thiocyanate was injected intravenously into the marginal vein of the ear of anesthetized animals as in the earlier experiments The results in two rabbits show that the sodium chloride tends to cause deeper and slightly quicker anesthesia with slower return to normal and must, according to the theory of Bancroft and Rutzler, act as a colloidal protein coagulant for the central nervous colloids, causing deeper anesthesia than with ether alone In the case of rabbit A 16 the return to normal with sodium chloride was almost twice as rapid as with ether alone Since this occurred several times with the sodium thiocyanate we cannot say that the

and thirty minutes. The experimental animal died following the intravenous injection of the sodium citrate. Sodium citrate probably caused overalkalinity of the blood with depression of the respiratory center.

*Experiment IX*—In this case the dosage of sodium thioevanate was reduced one half and the sodium amylal remained the same as before. Both rabbits recovered in three hours and thirty six minutes. This was probably due to the fact that the sodium thioevanate was too dilute for its action to be evidenced. The effects were those due to the sodium amylal alone since both animals reacted in the same manner and the experimental animal showed no toxic effects.

Throughout the eight experiments with sodium thioevanate (disregarding the one in which sodium citrate was used) the response to the needle prick was identical in each set, so that no appreciable difference in response could be demonstrated between the reaction of the control and that of the experimental animal. The respiration in none of the controls was as low as that recorded by Bancroft and Rutzler, which was 14 per minute after forty-two minutes of narcosis. The lowest respiration in our animals was twenty-four per minute after forty-two minutes of narcosis. Not one of the experimental animals arose of its own accord after the injection of the sodium thioevanate as Bancroft and Rutzler report occurred after the first injection and after each successive injection. In no case did the respiration of the experimental animal exceed that of the control more than two or three per minute. In three sets of experiments the respiration of the experimental animal was considerably slower than that of the control, which makes one suspect that the sodium thioevanate depresses the respiratory center rather than stimulates it.

It is interesting to note that our controls recovered to normal on the average of four hours and eight minutes of anesthesia. This almost checks with that of the one experiment of Bancroft and Rutzler which was four hours and fourteen minutes, but our animals never completely lost their lid reflexes nor had as low respiratory rates. Our experimental animals, with the exception of the fourth set, required from one third to twice as long to return to normal as did the controls, and three out of seven which received the injection of the 10 per cent solution of sodium thioevanate died within three days while none of the controls died. Those which did not die as well as those which did die, showed severe nervous symptoms which resulted in the inability to control properly the movements of the rear extremities. In several cases the thioevanate caused inability to move any of the legs or head in coordinate motion and the animals were unable to propel themselves even when conscious.

William Goldring and Herbert Chasis, who administered thioevanate to hypertension patients, found that 17 per cent developed toxic manifestations with hallucinations of sight, convulsive movements of the extremities, coma and even death in two cases. These toxic effects were unrelated to the amount of thioevanate administered and in some patients there was found to be little or no margin of safety between the toxic and therapeutically effective dose of thioevanate.

### III MORPHINE

The last group of experiments consisted in testing the power of sodium thioevanate as an antagonist against morphine. The dose of morphine sulphate

was 0.052 mg per gram of body weight, this being the same as that used by Bancroft and Rutzler

These workers reported one control animal compared with an experimental animal, and their results are briefly as follows

The control rabbit, weighing 2300 gm, received 0.12 gm of water solution of morphine subcutaneously. The animal was in deep narcosis after three hours and eight minutes at which time its respiration was 12 per minute. After five hours the respiration was only 18 per minute and the rabbit was still well under the influence of morphine.

At the start of the control experiment a companion rabbit was likewise narcotized. This animal was asleep in ten minutes. Five minutes later 3.8 cc of 10 per cent solution of sodium thiocyanate was injected intravenously, after which the rabbit was able to sit up although it was in a stupor. Later the rabbit appeared to be losing strength and 3 cc more of the thiocyanate solution were injected. This brought the strength back quickly and the respiration was 22 per minute after two hours and thirty-five minutes as compared to twelve of the control. After four hours the respiratory rate was thirty-eight per minute. Throughout the experiment this animal was on the border line of consciousness.

In my experiment four young rabbits were selected and subjected to the same experimental procedure as that used by Bancroft and Rutzler. Two were run as controls and received only morphine (0.052 mg with water solution per gram body weight) while the other two received morphine followed by intravenous injections of sodium thiocyanate. The first pair consisted of rabbit A 234 which was run as a control and given morphine only, and rabbit A 363 which was given morphine plus sodium thiocyanate.

*Rabbit A 234* weight 1950 gm (1) After one hour and eleven minutes the respiration was 10 per minute. (2) After two hours and twenty-two minutes the respiration was 16 per minute. (3) After four hours and thirty minutes the animal turned over on its abdomen when placed on its side. (4) After seven hours it was rapidly nearing normal and able to hop about slowly. (5) The following morning it was normal and able to eat.

*Rabbit A 363* weight 2010 gm received 3 cc NaCNS after fifteen minutes, 3 cc NaCNS after one hour and four minutes, and 1 cc after five hours and three minutes. (1) After one hour and ten minutes the respiration was 12 per minute. (2) After two hours and twenty-four minutes the respiration was 17 per minute. (3) After four hours and thirty minutes it tried to turn on its abdomen when turned on its side but was unable to do so. Instead its hind legs extended backwards spasmodically and seemed to be out of control and somewhat paralyzed. (4) After seven hours the rabbit was still unable to move or sit up. The hind legs were still extended and its breathing was forced. (5) The following morning it was still unable to get up. Its hind legs remained extended and appeared to be completely paralyzed. It lay as if it were dead except for its breathing and its head was rotated 60 degrees to the right. When it was picked up it made slow, forced locomotor motions with all its feet. It had diarrhea. (6) The second morning it was found dead.

The second pair consisted of rabbit A 119 which was the control and rabbit A 123 which was the experimental animal.

*Rabbit A 119* weight 1920 gm (1) After one hour and twelve minutes it was sleeping quietly. (2) After two hours the respiration was 15 per minute. (3) After four hours the respiration was 16 per minute. (4) After five hours the respiration was 17 per minute, and the rabbit was nearing normal. (5) The next morning the rabbit was normal and lively.

*Rabbit A 123* weight 2070 gm received 3 cc of NaCNS after fifteen minutes and 2.4 cc of NaCNS after one hour and twelve minutes. (1) After one hour and twenty minutes

or eight minutes after the second injection of 2.5 cc of sodium thiocyanate which was injected intravenously, the rabbit extended its hind legs backward and became very sensitive to slight shock or noise (2) After two hours the respiration was 15 per minute and the slightest touch caused rigid contractions which resembled strychnine contractions (3) After four hours the respiration was 24 per minute (4) After five hours the respiration was 20 per minute (5) The next morning the rabbit was free from morphine symptoms but its head was rotated 30 degrees to the left and it hopped in a circle to the right. A slight trip or jar caused the animal to jump and jerk spasmodically (6) The animal died during the following night

It will be noticed that in the preceding pair the experimental rabbit received a total of 7 cc of 10 per cent solution of sodium thiocyanate by the intravenous route. It weighed 2010 gm. In this last pair the experimental rabbit received only 5.4 cc by the same route although it weighed 2070 gm. This reduction however had no effect in the fatality of the dose as the animal was also found dead the second morning.

Since both of these animals died shortly after the experiment, it was decided that an animal should be subjected to the same dose of thioevanate without the morphine in order to determine whether or not the effects of thioevanate alone would cause death or whether the animal died as a result of the combined drugs. Rabbit A 73, weighing 2080 gm was given enough ether to relax the muscles and received the identical dosages injected at the same interval which were given to A 363 and totaling 7 cc. After the first injection the animal sat up when the ether mask was removed and hopped across the room as if it were normal. The second injection showed little effect but after the last was completed the rabbit was unable to hop normally. Its hind legs began to show signs of dragging and it bumped into objects in its path. Suddenly its front legs gave way and it sprawled on the floor unable to support its own weight. The fore legs remained extended to the sides as if the pectoral muscles had failed and the head and chest rested on the floor. The animal died during the night. It showed signs of diarrhea as did almost every rabbit which received either large or small doses of sodium thiocyanate throughout all of our experiments.

One more experiment was performed using morphine and thiocyanate but further decreasing the amount of the thiocyanate injected.

*Rabbit A 95* weight 1900 gm. Control animal (1) Received 0.098 gm morphine (2) After thirty two minutes of narcosis the respiration was 60 per minute (3) After one hour and twenty minutes the respiration was 67 per minute (4) After three hours and twenty minutes the animal was still dull and in a stupor (5) The next morning the rabbit showed complete recovery.

*Rabbit A 243* weight 1785 gm, was used as the experimental animal. It received a total of 3.9 cc of thiocyanate solution. (1) Injected subcutaneously 0.092 gm of morphine (2) After fifteen minutes of narcosis 2.4 cc of 10 per cent solution of sodium thiocyanate was injected intravenously into the marginal ear vein (3) After thirty two minutes of narcosis the respiration was 30 per minute (4) After one hour and three minutes 1.5 cc more of thiocyanate was injected (5) After one hour and twenty minutes respiration was 46 per minute (6) After three hours and twenty minutes the animal was up and hopped about but held its head with a 20 degree rotation to the right (7) The next morning the animal was still quite weak and would not eat during the day (8) The second morning the rabbit evidently had completely recovered. It was eating and quite active.

Although this rabbit did hop about a little sooner than the control, it showed signs of toxic effects for a day following the experiment and evidently barely escaped the fatal effects of the drug. The control had a respiratory rate through-

out the experiment which was from a third to a half again as rapid as that of the experimental rabbit. This showed depression of the respiratory center by the thiocyanate rather than a stimulation.

Of the three rabbits receiving the morphine and thiocyanate two died within forty-eight hours and the other barely escaped, although the dose was reduced, since it showed decided intoxication during the first twenty-four hours following the experiment.

#### CONCLUSIONS

1 The results obtained in experiments with sodium thiocyanate as an antagonist for ether did not corroborate the conclusions brought forth by Bancroft and Rutzler that thiocyanate ions antagonize the anesthetic action of ether. In a large majority of the ether experiments the return to normal of the lid reflexes and other manifestations required a longer time in these experimental animals than in their controls. In the quantities used seven out of seventeen rabbits which received the ether and thiocyanate died.

2 In experiments with sodium thiocyanate as an antagonist for sodium amytal it was found that the sodium thiocyanate did not shorten the long anesthesia but instead lengthened it, and decreased instead of increased the respiratory rate. Three of seven rabbits died.

3 In experiments with sodium thiocyanate as an antagonist for morphine, it was found that the morphine narcosis was not counteracted in any way, and that gradient reduction in the dosage of thiocyanate still proved fatal or toxic. Two of the three rabbits receiving the thiocyanate died.

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# MICROSCOPIC SLIDE PRECIPITATION TESTS FOR THE DIAGNOSIS AND EXCLUSION OF SYPHILIS WITH SORE FLUID\*†

## PRELIMINARY REPORT

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**B**ASED upon 329 tests of varied sensitivity with various quantities of 126 fluids from 94 venereal and 12 nonvenereal sores with control clinical observations blood tests, darkfield examinations and examinations of stained smears, the following microscopic slide precipitation tests for the diagnosis and exclusion of syphilis with sore fluid† are proposed

Into each of two open slide chambers, 11 mm. in diameter 0.025 cc sore fluid previously heated at 56° C for one-half hour is pipetted. Additional specimens may be similarly pipetted into other chambers upon the same slide.

Into one-half of the chambers a very small drop (about 0.004 cc) of diagnostic test emulsion is allowed to fall from a capillary pipette.

Into each of the other sore fluids a very small drop of exclusion test emulsion is allowed to fall from a capillary pipette.

The slide is rotated on a flat surface with moderate vigor for five minutes.

The results are examined at once through the microscope at a magnification of about 120 times (16 mm objective 12× ocular) with the light cut down as for the study of urinary sediments and recorded in terms of pluses according to the degree of clumping and the size of the clumps.

The principles of the sore fluid tests described above are the same as those for the tests with heated serum<sup>1, 2</sup>

### MATERIALS FOR THE MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS WITH SORE FLUID (SEE FIG 1)

1 *Sore Fluid*—The lesion is thoroughly cleansed with sterile distilled water until all the pus, necrotic material and any local medication is completely removed. After proper cleansing, the clean base of the lesion is touched with a sterile platinum loop and the material obtained is used for a darkfield examination. The metal end of a 10 cc Record syringe barrel (see Fig 1) is then adapted to the lesion and negative pressure produced with a small negative pressure pump (see Fig 1). The suction is allowed to continue until after the darkfield examination has been made and recorded and then it is terminated. The three or four drops of sore fluid in the barrel are allowed to run into a capillary glass pipette 5½ inches in length, 3 mm in diameter. The capillary pipette is

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†Sore fluids turbid with exudate or containing any appreciable amount of local medication are unsatisfactory for testing. Admixture with blood however even in considerable quantity does not make the specimen unsatisfactory for the tests.

next touched to the base of the sore for additional fluid. If necessary, further suction is applied and the fluid obtained transferred to the capillary pipette. One end of the pipette is now sealed over a Bunsen burner. A second darkfield examination is then made and smears are prepared for special staining. The initial cleansing of the sore cannot be stressed too greatly since the presence of local medication, pus or debris in nonsyphilitic sore fluid may cause the precipitation of the antigen emulsion.

The sore fluid in the pipette is centrifuged for thirty to forty-five minutes at high speed (about 2000 r p m) and the gross character of the fluid and the sediment noted. The pipette is then placed in a bath with water at a higher level than the top of the sore fluid and kept at 56° C for one-half hour. After this the pipette is filed and broken just above the sediment and the fluid is allowed to run into or is drawn into a 0.2 cc pipette graduated in 0.001 cc.

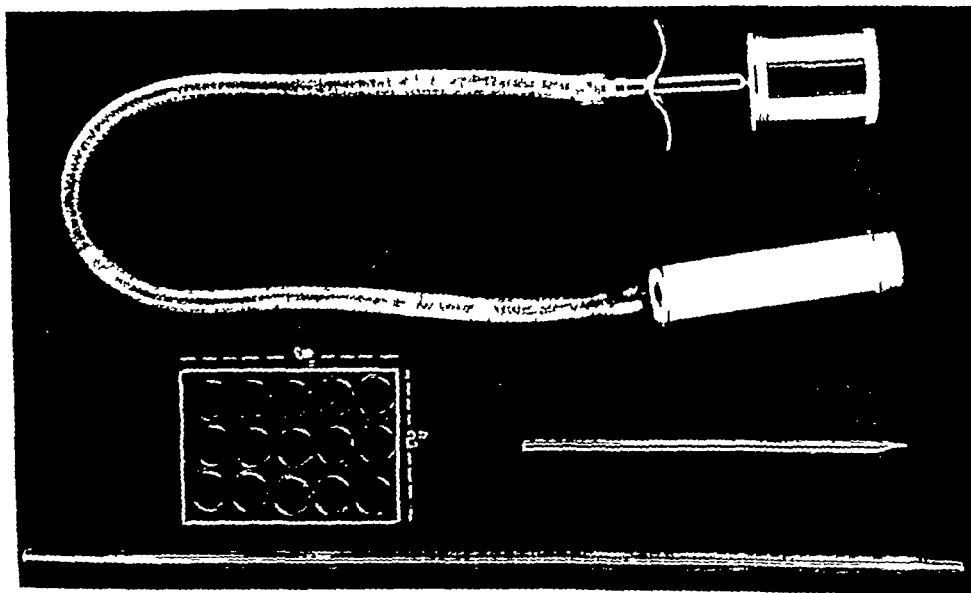


Fig. 1—Materials for microscopic slide precipitation tests for syphilis with sore fluid

The technique of obtaining and the preliminary handling of the sore fluid is much like that reported by Ellhott and Todd.<sup>3</sup>

2. *Glassware*—The pipette used to deliver the proper quantity of sore fluid is a 0.2 cc pipette graduated in 0.001 cc.

The pipettes for the antigen emulsion are Wright pipettes made from 6 to 10 mm glass tubing drawn out into a very fine tube (about 1/4 mm in diameter).

Ordinary finely graduated 0.2 cc, 1 cc and 10 cc pipettes are employed in the preparation of the antigen emulsions described below.

The antigen emulsions are prepared in ordinary cork-stoppered narrow mouthed 1 ounce bottles.

Microscopic slides 2 by 3 inches as purchased are rubbed on both sides with Bon Ami paste (prepared by allowing a cake of Bon Ami to remain in sufficient warm water to cover it for twelve hours or more). The paste keeps well but may

B Total Nonvenereal Cases		12 as follows
1	Impetigo	2
2	Vincent infection	1
3	Molluscum contagiosum	1
4	Furuncle	1
5	Infected scabetic lesion	1
6	Herpes	1
7	Actinomyces	1
8	Erythema multiforme	1
9	Rodent ulcer	1
10	Indurated genital lesion, 1 year's duration (undiagnosed)	1
11	Indurated genital lesion, 14 years' duration (undiagnosed)	1

Table I shows the results of active syphilitic sore fluid and blood examinations in the active syphilitic cases

In each case, a record was kept of the clinical data including the character of the sore and other related structures, the time of appearance, the times of exposure, the antiseptic if any applied, the reaction of the patient at the time the sore fluid was obtained, etc. In each instance the gross and microscopic character of the sore fluid was noted. A darkfield examination or an examination of a smear of the sore fluid stained by the Fontana method was made in each instance, usually both. When indicated smears were prepared by special stains and examined for Ducrey's bacillus and Donovan bodies. To control the sore fluid results blood withdrawn from an arm vein at the time the sore fluid was obtained was tested for syphilis by the microscopic slide precipitation and Wassermann methods. In the majority of cases a spinal fluid examination including a Wassermann test was likewise made. All of the patients were followed for a sufficient period of time to rule out any masked infection.

In the beginning of the study fluid was obtained from the sore by using firm pressure of gloved fingers on both sides of it and was allowed to run into a capillary pipette. This method caused much pain and discomfort to the patient and required a long time to obtain more than a drop or two. In the course of time, the technic described above was developed by which the patient was caused little or no discomfort and the fluids obtained for testing were thoroughly satisfactory in quantity and character.

At first, 0.01 cc of sore fluid was employed in the open slide precipitation tests. With the development of a satisfactory method of obtaining larger quantities of fluid 0.025 and 0.05 cc were used. When 0.01 cc was tested, a similar quantity of antigen emulsion was added. The microscopic slide precipitation tests with blood obtained at the same time gave so much stronger results with the same emulsions in positive cases that it was considered advisable to change the conditions of the sore fluid test. By employing 0.025 and 0.05 cc of sore fluid and by the use of very small or small drops of antigen emulsion equal to about one-sixth the quantity of sore fluid, results more comparable with those of the blood were obtained. In the great majority of instances however with conditions quite comparable and with the same (diagnostic blood test) antigen emulsion, the blood gave decidedly stronger reactions than did the sore fluid. For this reason the use of the more sensitive emulsion described above for the diagnosis of syphilis with sore fluid is justified. Likewise, with condi-

It is held at an angle again, and the proper amount of antigen is pipetted against the side of the neck of the bottle from a finely graduated pipette

The bottle is promptly stoppered with a cork and shaken vigorously (the fluid thrown from bottom to cork and back) for one minute

Lastly, the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken less vigorously than previously for one minute

The emulsion, when examined under the microscope at a magnification of about 120 times, shows numerous very fine particles, but no clumps whatever

#### *For Diagnostic Test*

One cubic centimeter or more of the emulsion in a narrow test tube (about 12 mm inside diameter) is placed in a water bath at 35° C for fifteen minutes. The emulsion as soon as heated is ready for use

#### *For Exclusion Test*

Two cubic centimeters of the emulsion in a narrow test tube (about 12 mm inside diameter) is placed in a water bath at 56° C for fifteen minutes, after which it is poured into a 3 by 1 inch tube and centrifuged for fifteen minutes (eighth setting of Rheostat of International Equipment Company Centrifuge, size 1, type SB about 2000 r p m unloaded)

After centrifugation, the fluid is decanted and with the tube inverted, the inside is dried with a cloth almost to the level of the sediment

To the sediment 3 c.c. of 0.85 per cent sodium chloride solution is added

The sediment is distributed throughout the fluid by shaking and then poured into a narrow tube for use

These emulsions, kept at room temperature, are satisfactory for at least twenty-four hours after preparation

#### COMMENT

The majority of sore fluids were obtained from patients in the venereal wards of City Hospital and Lakeside Hospital through the kindness of H. N. Cole. The remainder were obtained from patients attending Mount Sinai Hospital and Charity Hospital. Those from the latter hospital were obtained through the courtesy of C. G. LaRocco. A few were obtained from patients of S. L. Bernstein and J. R. Breitbart and thanks are due for their kind cooperation.

A summary in brief of the sore fluids examined follows

Total Specimens Examined	126
Total Tests (with various quantities of sore fluid and with emulsions of varying sensitivity)	329
Total Cases	106
A Total Venereal Cases	94 as follows
1 Active syphilitic	36
(a) Primary	18 Genital chancres (1 associated with chancroid)
(b) Secondary	17 Genital or anal condylomata or papillomas
(c) Congenital	1 Bulla
2 Chancroid	
3 Granuloma inguinale	54
4 Lymphogranuloma inguinale	3
	1

B Total Nonvenereal Cases	12 as follows
1 Impetigo	2
2 Vincent infection	1
3 Molluscum contagiosum	1
4 Furuncle	1
5 Infected scabetic lesion	1
6 Herpes	1
7 Actinomycosis	1
8 Erythema multiforme	1
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# LABORATORY METHODS

## ELEMENTAL IODINE\*

### A NEW TECHNIC FOR THE EVALUATION OF ITS FUNGICIDAL PROPERTIES

ALBERT STRICKLER, M D , PHILADELPHIA, PA

#### INTRODUCTION

IN MODERN medicine new therapeutic suggestions do not rest on empiricism alone, but are founded on facts revealed through investigative laboratory methods. The efficiency of iodine as a fungicide has been known and its place among this class of remedies is quite secure. Empiricism first and subsequently favorable clinical results have been responsible for faith in iodine. Although it had long been known that iodine vaporized very readily, application of iodine vapor to the skin has not been medically used, but instead, various preparations of iodine as the tincture of iodine, Lugol's solution and the official Unguentum Iodi have been introduced and popularized. Theoretically, these combinations do not add strength to the iodine, but on the contrary, probably lessen its efficiency. In the tincture of iodine, the addition of the alcohol hardens tissues by absorbing the water and precipitating the proteins,<sup>1</sup> a condition which should interfere with the penetration and activity of the iodine. The strength of the tincture of iodine (U S P) is only 7 per cent. The iodine ointments are weak insofar as iodine activity is concerned. They do not react readily with proteins, as they give up their iodine slowly, owing to previous chemical combination of the iodine with the bases of the ointment preparations. The official iodine ointment consists of 4 per cent each of iodine and potassium iodide in glycerin and wool fat.

It is evident that both theoretically and practically, the commonly used iodine compounds are not ideal fungicides. These preparations of iodine do not utilize 100 per cent of this agent, the tincture being a 7 per cent preparation and the official ointment a 4 per cent combination. In the tincture, as previously stated the alcohol tends to limit the penetrability of the iodine and increases its tendency for skin irritation, while in the official ointment, its combination with fatty bases tends to decrease markedly iodine activity. The ideal fungicide should possess the following qualities: penetration, prolonged fungicidal activity, minimum reaction with the cutaneous tissues, and specific chemotaxis for the fungus protoplasm.

The introduction of chemotherapeutic methods has opened a new avenue for the exact evaluation of the old fungicidal agents and has offered hope for the discovery of the ideal remedy.

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## PREVIOUS FUNGICIDAL EXPERIMENT

The earliest of these attempts was the investigation of Schamberg and Kolmer<sup>2</sup> in which the fungistatic and fungicidal activity of various dyes and medicaments were studied. Their method consisted of placing in each test tube 4 cc of Sabouraud's maltose agar titrated to 1 per cent acid to phenolphthalein. The dyes and medicaments were prepared in 1:100 solution or suspension in sterile distilled water and higher dilutions were prepared in sterile test tubes in amounts of 1 cc. The agar and solution or suspension of dye or medicament were mixed, slanted and allowed to harden. These tubes were then seeded with the respective cultures of the *Trichophyton rosaceum* and *Microsporon audouinii*, and *Achorion Schoenleini*. The tubes were kept at room temperature. The results were read at weekly intervals for six to eight weeks.

In this investigation twenty-one compounds were studied including iodine, mercuric chloride, calomel, salicylic acid, benzoic acid, sodium hyposulphite, etc. As a result of this study, the authors concluded that "Iodine transcends all other medicaments in restraining the growth of certain moulds but in aqueous solution it is inferior to certain other drugs in killing them." The strength of the iodine used in these experiments was 1:2000 of a 2 per cent solution (2 gm iodine crystals, 5 gm potassium iodide and 100 cc water) = 1:100,000 of pure iodine.

The pioneer researches of Schamberg and Kolmer seemed to focus attention upon iodine and its compounds as offering the most fertile basis for the uncovering of the ideal fungicidal agent. Following these investigations came the work of Shalit and Highman<sup>3</sup> who proposed the use of a compound of iodine first described by Horton in 1888 and known as tetraiodomethenamine ( $(CH_2)_6N_4I_4$ ). Being an unstable iodine compound, it was found capable of giving off free iodine vapor very much after the manner of iodine crystals only more slowly. The reasons advanced by the author for proposing this iodine compound were that, "Iodine as a fungicide should be most efficacious in the treatment of patients who have mycotic dermatoses, not alone for its high fungicidal coefficient, but also because iodine extremely volatile at ordinary temperature, may be fed to the skin as a gas and as such has a fair measure of penetrability. A constant stream of iodine acting over a long period of time, reaching the skin in fungicidal strength yet not irritating the skin is a desideratum." Here we have for the first time an inkling that iodine vapor applied to the skin may possess superior fungicidal properties.

In order to determine experimentally the relative fungicidal efficiency of tetraiodomethenamine and of iodine, the authors set up a number of tests as follows. Varying concentrations of tetraiodomethenamine and of iodine were added by constant volume to Sabouraud's media and these were slanted and hardened before being seeded with cultures of the *Microsporon lanosum*. They were incubated at room temperature. To render both the free iodine and the tetraiodomethenamine water soluble, potassium iodide was added to each of the solutions. The growths were examined at the end of two weeks. These experiments seemed to show the superiority of the tetraiodomethenamine over iodine, inasmuch as the iodine in both a 1:40,000 and a 1:20,000 dilution

tailed to inhibit the fungus growth, while in those same dilutions, tetraiodomethenamine did have fungistatic action

Another experiment devised by the authors for study of the fungistatic action of these two preparations consisted in taking bits of masses of fungi about pea size, soaking them in 1 cc of an aqueous solution of iodine and of tetraiodomethenamine respectively for forty-eight hours, seeding on appropriate media and growing at room temperature. These experiments showed

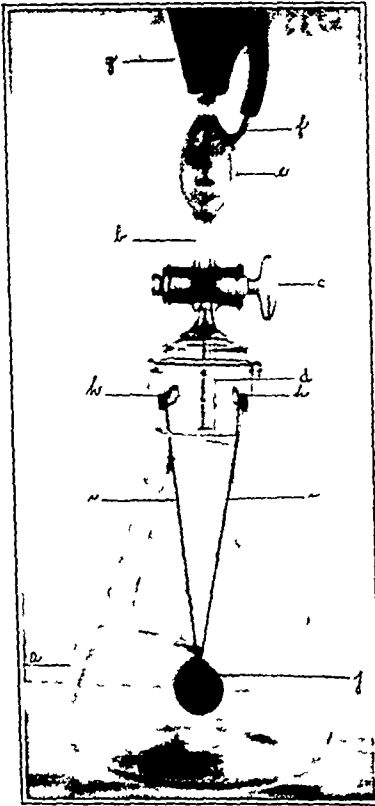


Fig 1

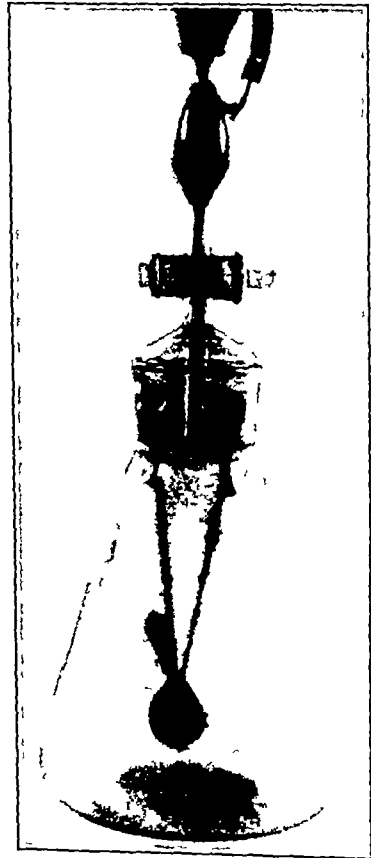


Fig 2

Fig 1—The Strickler Borneman apparatus. *a* Pyrex flask, *b* neck ground to fit airtight with inverted glass cup, *c* stop cock, *d* central glass tube, *e* bulb-like expansion, *f* hollow glass side arm, *g* vacuum vaporizer, *h* two glass hooks, *i* threads, *j* collodion etc.

Fig 2—The Strickler Borneman Apparatus after iodine vapor treatment

equal fungistatic powers for both the aqueous iodine plus potassium iodide combination and for the tetraiodomethenamine plus potassium iodide mixture

The conservative interpretation placed upon the above experiments is an acknowledgement of the marked difference between the iodine laboratory experiments and application of this remedy to the human skin in disease

'The Strickler Borneman Apparatus' Description. Clinical use of "The Renal Vacuum Vaporizer" with iodine as the medicinal agent prompted the author to devise a laboratory method for testing the fungicidal powers of iodine vapor in a manner comparable to its actual use on the human diseased skin

To meet this condition "The Strickler-Boinemann Apparatus" was devised. It consists of a pyrex flask (a) of about 1 liter capacity with the neck so ground as to fit air-tight with the inverted glass cup part (b) provided with a stop-cock (c). This inverted cup-shaped portion has a central glass tube (d) throughout its length for the passage of the iodine vapor, it also houses the stop-cock. The upper terminus of the cup-shaped portion consists of a bulb like expansion (e) with a hollow glass side arm (f) so arranged as to fit "The Henal Vacuum Vaporizer" (g). On the inner surface of the cup-shaped portion (b) and as close to the neck of the flask (a) as possible there are two glass hooks (h) one in each side to hold the threads (i) which suspend the collodion sac (j) loaded with the culture to be tested. Before each experiment a small amount of sterile petrolatum is applied to the ground fitting surfaces of "a" and "b" to insure air-tight contact.

#### TECHNIC

Collodion sacs were made by pouring several cubic centimeters of Merck's flexible collodion into large test tubes and distributing evenly over the surface. The test tubes were inverted to permit escape of the excess collodion making possible preparations of sacs of fairly uniform thickness. After allowing the collodion solvent (alcohol ether) to evaporate spontaneously the sacs were immersed in water to facilitate their removal from the tubes. Each sac was tested for leaks just prior to use to avoid the possibility of iodine vapor passing through small holes. The average thickness of the sacs was 0.01 to 0.02 mm. Tests with "The Henal Vacuum Vaporizer" showed that 0.0175 gm of iodine were delivered on each application and that two such applications were required to fill the flask with vapor.

The medium for growing the ringworm fungi consisted of peptone (French) 10 gm, crude dextrose 40 gm, water 1 liter (Weidman's Pennsylvania Medium without agar). The fungi were grown in this medium for approximately two weeks. Five cubic centimeters of the suspension of the organism tested was placed in a collodion sac the open end of which was tied with a sterile string and the end sealed with fresh collodion so that no vapor could penetrate except through the walls of the sac. The constant volume of suspension used made the exposed surface fairly constant so that differences occurring could not be due to variation in the surface exposed to the iodine vapor. Tests with the sac filled with suspension of the organism (ringworm fungus) and allowed to stand for one hour showed that the sac itself had neither lethal nor inhibitory effects upon the fungus in the absence of iodine vapor.

In conducting these experiments, the sealed collodion sacs containing suspension of the fungus tested were suspended in "The Strickler-Boinemann Apparatus" and subjected to two applications of iodine vapor by means of "The Henal Vacuum Vaporizer" using a negative pressure of 15 cm. After permitting the sacs to remain in the iodine atmosphere for varying periods of time they were removed and opened. Samples were obtained with sterile pipettes and transferred to culture tubes containing Weidman's Pennsylvania Medium. These were observed for varying periods of time as detailed in the schedules below. The control cultures were removed before subjection to iodine vapor.

TABLE I

FUNGISTATIC AND FUNGICIDAL RESULTS WITH IODINE VAPOR USING "THE HENAL VACUUM VAPORIZER" AND "THE STRICKLER BORNEMAN APPARATUS"

NAME OF FUNGUS	DATE OF EXPERIMENT	CONTROL	DURATION OF IODINE VAPOR EXPOSURE			
			5 MIN	15 MIN	30 MIN	60 MIN
<i>Aspergillus Niger</i>	8/10	8/11 growth			8/11 growth	8/11 less growth than 30 min
<i>Trichophyton gypsum pleomorphic</i>	8/11	8/17 growth	8/17 growth	8/17 no growth	8/17 no growth	
<i>Cryptococcus castellanei</i>	8/12	8/17 growth	8/17 growth	8/17 growth	8/17 growth	
<i>Microsporon audouinii</i>	8/14	8/17 growth		8/17 no growth	8/17 no growth	8/17 no growth
<i>Epidermophyton inguinale</i>	8/14	8/17 growth		8/17 growth	8/17 no growth	8/17 no growth
<i>Trichophyton asteroides</i>	8/20	8/26 growth		8/26 no growth	8/26 no growth	8/26 no growth

These cultures were observed for 5 days then formalized

Recognition of the possibility of delayed growth prompted repetition of these experiments In Table II the cultures were observed for twenty-two days

TABLE II

FUNGICIDAL RESULTS WITH IODINE VAPOR USING "THE HENAL VACUUM VAPORIZER" AND "THE STRICKLER BORNEMAN APPARATUS"

NAME OF FUNGUS	CONTROL	TIME OF EXPOSURE AND RESULTS	
		30 MINUTES	60 MINUTES
<i>Microsporon audouinii</i>			
began growth	3rd day	5th day	no growth
reached maximum	7th day	21st day	no growth
<i>Epidermophyton inguinale</i>			
began growth	5th day	no growth	no growth
reached maximum	13th day	no growth	no growth
<i>Trichophyton asteroides</i>			
began growth	3rd day	no growth	no growth
reached maximum	12th day	no growth	no growth

Figs 3 and 4 depict the results of exposure of ringworm cultures to iodine vapor in "The Strickler Borneman Apparatus" after reculture

Desire to determine whether iodine crystals as such, or, as iodine vaporized, were responsible for the results obtained in the above experiments prompted the setting up of another series of fungicidal tests Three different strains of ringworm fungi were grown in Weidman's Pennsylvania Media without agar for two weeks they were the *Microsporon audouinii*, the *Trichophyton asteroides*, and *Epidermophyton interdigitale* To each of the collodion sacs containing 5 cc of the above ringworm culture suspensions iodine crystals in the following respective amounts were added i.e 0.00103 gm 0.00216 gm, and 0.00386 gm These amounts of iodine were determined as present within the collodion sacs

after their exposure to iodine vapor for 15 min, 30 min, and 60 min respectively, using the Strickler-Borneman apparatus and the Henal Vacuum Vaporizer. The contents of the sacs were thoroughly agitated and mixed and kept at room temperature for fifteen minutes, thirty minutes and sixty minutes, respectively. The amounts of iodine and the time exposures were identical with those used in the conduct of "The Henal Vacuum Vaporizer" and "The Strickler-Borneman Apparatus" fungicidal tests. At the expiration of the respective time limits, each of the cultures was transplanted by placing a few drops of the iodine treated suspension on Pennsylvania agar slants. Daily observations were made and the first indications of growth appeared from the third to the seventh



Fig 3



Fig 4

Fig 3—*Microsporon audouinii* 1 Control 2 delayed growth Iodine vapor fifteen minutes

Fig 4—*Microsporon audouinii* 1 Controls 2, iodine vapor thirty minutes 3 iodine vapor sixty minutes

day. This observation period lasted three weeks. The results showed that the growth in the iodine treated cultures almost equaled that of the control, so that iodine crystals per se, as used in the above manner seem to have failed to retard growth of the ringworm fungi.

#### COMMENT

The experiments here cited warrant the following comments:

1 "The Strickler-Borneman Apparatus" and the technic suggested, offer a new method of testing the fungicidal and fungistatic action of iodine vapor, through interposition of a nonabsorbable membrane between the fungi and

the medicament and through use of the agent tested in the form identical with that applied to the human skin. No doubt the same technic could be applied to other vapors and gases. The identical technic has been used for testing the bacteriocidal properties of iodine vapor, and upon this phase, a report will be made shortly.

2 The laboratory experiments proposed simulated closely conditions of treatment adopted for epidermophytosis and for tinea tonsurans in the human being.

3 Proof has been furnished of the remarkable fungicidal properties of iodine vapor, confirming the suggestive conclusions of other authors.

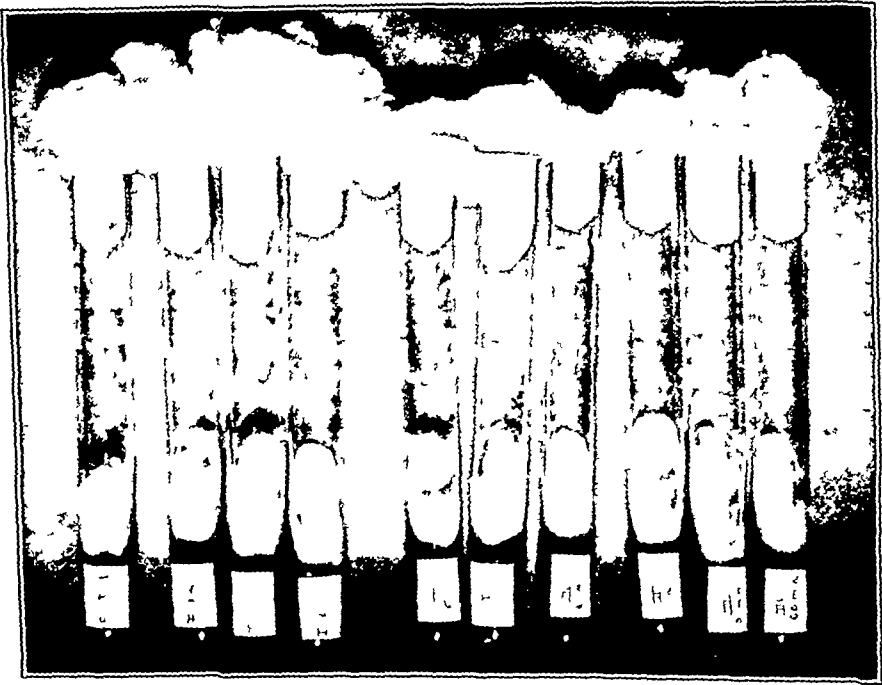


Fig. 5.—*Epidermophyton interdigitale* agar cultures after treatment with iodine crystals. Note: Growth in treated tubes practically equals growth in control tube.

4 The cultures observed for only five days after treatment with the iodine vapor gave, in the main, identical results as those observed for twenty-two days, showing absence of delayed growth and proving that iodine vapor really possesses fungicidal action.

5 Experiments are now being conducted with the thought of formulating means for prolonging iodine activity and for increasing its chemiotropism for the ringworm fungi. As this work is far advanced a report of these findings will be released in the very near future.

#### ADDENDUM

Since writing this paper a contribution by Sehamberg and his associates has appeared in the December 1931 issue of *The Archives of Dermatology* in which they state that metallic iodine appears to be the strongest present day

fungicide They based this statement on certain experiments conducted with Lugol's solution

The author wishes to express his appreciation to Mr George Borneman of Millville, New Jersey for his efforts in the construction of "The Strickler Borneman Apparatus "

The technical part of this investigation was carried out by Frederick Summerill, M.D., and Philip D Adams, M S., of the Research staff of The Skin and Cancer Hospital of Philadelphia to whom the author wishes to express his appreciation

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806 PINE STREET

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### THE WIDAL TEST IN TUBERCULOSIS\*

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**D**URING the past year two instances have come to my attention in which a positive macroscopic agglutination test was obtained for typhoid fever in a titer (1:320) usually regarded as diagnostic for that disease, although the patients had no clinical symptoms of typhoid fever They did however, have tuberculosis In one case the infection was pulmonary in the other, renal One patient gave no history of either typhoid fever or antityphoid vaccination The other patient had been vaccinated twelve years previously but there was no history of typhoid fever On inquiry among clinicians primarily interested in tuberculosis, another instance of this kind was found and the general impression appeared to be that positive Widal tests were occasionally encountered in tuberculous infection, and but little attention was paid to them

In 1923, Hull and Henkes<sup>1</sup> reported positive microscopic Widal tests (serum dilution 1-40) in 53 of 100 specimens of blood from early tuberculosis and in 1 of 13 advanced cases The macroscopic tests on 12 specimens were negative although 8 of these were positive by the microscopic method Hull and Henkes<sup>2</sup> also have studied agglutination of B dysenteriae Flexner by blood serum from persons in the incipient stage of tuberculosis, and obtained 75 per cent positive results They state that no agglutination was obtained with blood from cases of advanced tuberculosis or from normal individuals Kilduffe and Hersohn<sup>3</sup> have made similar observations Since the completions of my studies Madgwick and Partner<sup>4</sup> have published a paper dealing with serum reactions in tuberculosis

It seemed desirable to make a further study of this reaction, particularly in the light of the recent work of Felix<sup>5</sup> and Craigie,<sup>6</sup> and Eldering and Larkum<sup>7</sup> with the so-called "H" and "O" types of agglutination Since my two instances of "false" positives were in persons having advanced tuberculosis, this type of in-

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\*Department of Health  
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fection was studied. Blood was obtained from 61 hospital patients,\* all adults, 50 men and 11 women. The Kahn test was positive in 4 persons, negative in 53, not made in 4. In 7 instances there was a definite history of typhoid fever, the most recent being 11 years ago. Nine had received antityphoid vaccination.

The agglutination tests were made macroscopically, using serum dilutions ranging from 1-20 to 1-320. Two formalized typhoid antigens were used. One was a Rawlings strain. This strain is commonly used for Widal tests in diagnostic laboratories. Our culture gave an "H" or flocculent type of agglutination. The other antigen was obtained through the courtesy of Dr. N. W. Larkum of the Michigan State Department of Health and was marked "O" 901<sup>s</sup>. This culture was rich in "O" antigen and gave the characteristic "O" type agglutination. In addition, B paratyphosum A and B antigens were used.

No agglutination was obtained in any serum with either of the paratyphoid cultures. The "H" antigen (Rawlings strain) and the "O" antigen gave results as shown in Table I. The other 53 serums did not agglutinate either the "H" or "O" antigens. Kahn tests on the eight serums which agglutinated the "H" type culture were all negative.

TABLE I

TITER AGAINST ANTIGEN			HISTORY OF	
			TYPHOID FEVER	ANTITYPHOID VACCINATION
"H"		"O"		
1	20	none	Yes	Yes
1	40	none	Yes	No
1	40	none	No	No
1	40	none	No	?
1	80	none	No	No
1	80	none	No	No
1	160	none	No	Yes
1	160	none	No	Yes

It is apparent that the Rawlings strain of the typhoid bacillus may give the flocculent type of agglutination in the blood serum of patients ill with tuberculosis, but having no clinical signs of typhoid fever. In some instances (4 of 61 in this series), the titer fell in a range usually considered of diagnostic significance. There was no relation in this small series between such titer and the history of an attack of typhoid fever or of antityphoid vaccination. It is admitted that previous typhoid infection in any group cannot be absolutely ruled out. Since all of the serums which agglutinated the "H" antigen failed to agglutinate the "O" antigen, this latter type of agglutination must be considered in interpreting the results of Widal tests in advanced tuberculosis. These data emphasize the relative value of the two antigens in ruling out typhoid infection as a complication in that disease. It may not be out of place to point out that anomalous serologic results have not infrequently been reported in connection with tuberculosis. False positive Wassermann tests have been encountered although Kolmer<sup>1</sup> believes the results are due to technic, agglutination reactions in blood serum with typhoid and dysentery bacilli have been observed, and Pinner and

\*The specimens of blood and histories of patients were obtained through the courtesy of Dr. C. E. of the Herman Kiefer Hospital. The agglutination tests were made by Marie-Louise Lambert.



Voldrich have recently reported the presence of agglutinins for *Staphylococcus aureus* in tuberculous effusions<sup>10</sup>

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### THE PREGNANCY TEST IN RABBITS\*

#### OPERATIVE DEMONSTRATION OF THE OVARIES WITH MINIMAL ANTISEPSIS

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RABBITS have entirely replaced mice for the pregnancy test in our laboratories. It is the purpose of this paper to describe our operative procedure with minimal preparation and antiseptics for demonstrating the ovaries. This makes it possible to use one rabbit at least three times. The cost of a test however, is actually a little more than one third of the price of a rabbit because of the chemicals and suture material used. The saving is considerable in an institution doing a large number of tests.

With the rabbits under ether anesthesia laparotomies were done at first. We had a high mortality in spite of moderately careful asepsis. This was undoubtedly due to the transabdominal approach to the ovaries requiring a large incision with a consequent exposure and infection of the peritoneum. Since the ovaries lie against the back of the rabbit at the ends of the uterine horns, the idea of an approach through the back seemed logical. This method has been used successfully in performing oophorectomies on mice.

#### TECHNIC

The Friedman technique<sup>1</sup> was followed in administering the urine to be tested. Rabbits used were females three to four months old. Occasionally, however, we have used adult females which had been isolated singly for one month. The animals were given 7 to 10 cc of a filtered morning specimen

\*From the Worcester City Hospital Pathological Laboratory and the Medical Arts Clinical Laboratory.

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<sup>1</sup>Friedman, M. H. Mechanism of Ovulation in Rabbit. Ovulation Produced by Injection of Urine From Pregnant Woman. *Am J Physiol* 90: 617-22, 1929.

of urine through the marginal ear vein. Care was taken to make sure that the urine was not below 100m temperature. We had one fatality after administering urine soon after it was taken from the ice box. A minimal period of thirty-six hours was allowed to elapse before examining the ovaries.

*Preparation*—The rabbit, under ether anesthesia, is placed in the position normally assumed by the rabbit when at rest. The lower back is covered with tincture of iodine which is rubbed into the hair with a sponge. The hair was cut with scissors on some rabbits, and it was found easier to suture the skin.

The instruments necessary are a scalpel, tooth forceps, a hook, scissors, needle and needle holder, and a No 1 catgut suture (Fig 1). These are placed in a dish of 70 per cent alcohol. The operator wearing rubber gloves rinses

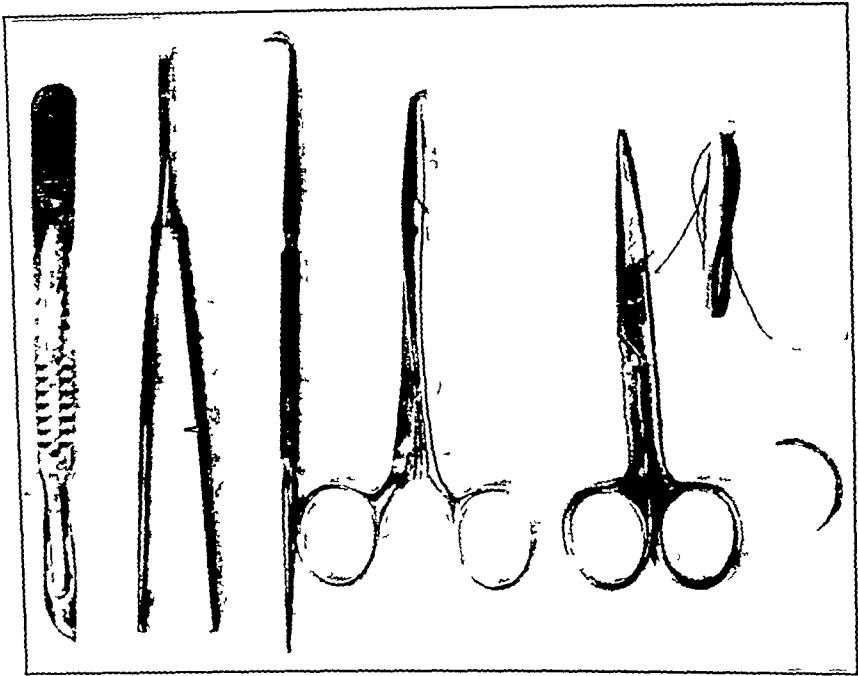


Fig 1—Instruments used in operation

his finger tips in the alcohol. (The alcohol is filtered after the operation and is used again.)

*Operative Procedure*—The operator locates the fifth vertebra above the juncture of the femur with the coxal or pelvic bone. A 4 cm longitudinal incision is made in the midline and should be bisected by the above mentioned vertebra. The incision is carried completely through the skin which is then readily separated from the fascia and muscle layer beneath. Because of the looseness of the rabbit skin the single incision serves for the examination of both ovaries.

The skin incision is now pulled to one side until the white line of the lumbodorsal fascia is seen. This is usually about 2 cm from the midline of the vertebral column and is just lateral to the thick sacrospinalis muscle. A 2 cm incision is made through the lumbodorsal fascia. The intestines are then

seen through the parietal peritoneum. The peritoneum is picked up with forceps and is cut allowing entrance into the abdominal cavity. The hook is then inserted and the ovary is brought into view. The ovary is usually found medial to the incision. The hook sometimes engages with the horn of the uterus or with the tube, but in either case the ovary is readily found. In a careful operation bleeding is not usually encountered. The ovary is inspected (Fig 2), and then dropped back into the peritoneal cavity. The same procedure is repeated on the other side through the one skin incision by drawing it over to the proper position. If the first ovary is definitely positive, the second ovary is not examined.

The incisions in the fascia are not sutured for two reasons. First because of the difficulty in entering at subsequent operations and second, because of the time involved. The rabbits thus far have not had a hernia through these openings. The skin is sutured with No. 1 plain catgut, and alcohol is soaked into the suture line.



Fig 2—Operative wound with ovary exposed on hook. Rabbit in prone position.

In early subsequent operations the catgut is not absorbed, and the healing wound is cut out with scissors. Granulation tissue is found in the old fascia wounds and bleeds slightly. Sometimes the uterine horn or ovary will be involved in the granulation tissue. Intestines have never been found in the granulation tissue.

#### DISCUSSION

Some operations were performed after using one gram of sodium amytal intravenously. This acts as an effective anesthetic on rabbits, and they remain unconscious for five to six hours. It is not used regularly, however, because of the cost.

In a series of 61 operations on 35 rabbits we have had 5 deaths from peritonitis. Two deaths occurred after three operations, and three occurred after one operation. In general the rabbits cannot be used for more than three tests because of thrombosis of ear veins or granulation tissue in the wounds of the lumbodorsal fascia.

The test has been repeated as early as three days following an operation which revealed negative ovaries. A subsequent paper will deal with the results of repeated tests in both negative and positive rabbits.

The defects in the asepsis are fully recognized. It is, therefore, nothing more than partial antisepsis. As such we have had a low mortality and feel that the procedure is worth continuing. Even with the operative time of five to seven minutes plus the cost of materials there is a definite saving on each test.

An operative procedure on rabbits is helpful when one desires a diagnosis of pregnancy at the end of fifteen hours. The diagnosis can then be confirmed by examining the ovaries of the rabbit again at the end of thirty-six hours, and thus save injecting a second rabbit.

The question arises as to the disposal of rabbits after the third operation. We have exchanged three used rabbits for one fresh rabbit. The dealer is now trying to breed them.

#### SUMMARY

A simple operative procedure is described for demonstrating rabbits' ovaries. The operation is used primarily in carrying out the pregnancy test in rabbits. The cost of the test is reduced almost two-thirds, and the extensive preparation and asepsis of a laparotomy are not necessary.

The authors wish to express appreciation of the technical assistance of Matthew Carrigan.

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## BACTERIOLOGIC TESTING OF CATGUT SUTURES\*

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IN CARRYING out most bacteriologic examinations, the object to be attained is the demonstration of the presence or the absence of some particular species of bacteria. In the case of surgical sutures, however, the purpose of the bacteriologic tests is to prove the entire absence of all forms of bacteria, absolute sterility. Such a procedure must, of necessity, involve a highly specialized technic carried out under the most rigid conditions.

#### CONTAMINATED COMMERCIAL CATGUT SUTURES

Competent bacteriologists, after making extensive bacteriologic examinations over a period of years of commercial catgut sutures marketed in various countries have reported a high percentage of contaminated sutures. Thus, in England, Bulloch<sup>1</sup> found more than 75 per cent of the sutures which he examined of eight different manufacturers to be infected with living bacteria. In Germany, Knorr<sup>2</sup> found that at least 80 per cent of the commercial catgut sutures that he examined contained both pathogenic and nonpathogenic bacteria. In the United States, Meleney and Chatfield<sup>3</sup> examined sutures from seventeen different manufacturers and found that the products of seven manufacturers contained living bacteria.

Because of the large percentage of contaminated commercial catgut sutures reported in various countries by competent bacteriologists, the careful bacteriologic testing of catgut sutures by an approved method and with adequate con-

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tiols is of paramount importance to bacteriologists as well as to the surgical profession

#### THE PROPOSED STANDARD BACTERIOLOGIC TEST FOR CATGUT

As a result of their three-year study of all existing bacteriologic methods of testing catgut sutures, Meleney and Chatfield devised and proposed what they termed a standard test for effectively determining the sterility of catgut. Some of the essential steps in this technique are similar to those devised six years ago by Dr Benjamin White, and successfully used by him during the five-year period prior to the time the standard test was proposed. However, this standard test possesses distinct improvements and advantages over the methods previously used for testing catgut.

*Anaerobiosis*—The anaerobic culture medium for the standard test is that devised by Novy<sup>4</sup> in 1893 and so successfully used by him since that time for anaerobic culture work. The anaerobic seal that was recommended by Meleney and Chatfield was originally devised by Hall<sup>5</sup> in 1929 in connection with the study of anaerobes. He coined the name "vaspar," and recommended it as a seal which effectively prevents evaporation as well as the reabsorption of oxygen. Hall also found that the white mineral oil, so widely used as a seal, is inefficient because it is permeable for oxygen, and his findings were confirmed by Meleney and Chatfield.

*Incubation Period*—For several years the incubation period recommended and used by Dr Benjamin White for testing surgical sutures was fourteen days. This period of time had always proved adequate, because it provided ample opportunity for even small numbers of bacteria that might be present in the catgut to adapt themselves to the new environment, and for the culture medium to reach a reduction potential satisfactory for their growth. The Therapeutic Substances (Catgut) Regulations of 1930 adopted by Great Britain specify a period of twelve days for incubating the tubes of culture medium containing the catgut sutures. However, Meleney and Chatfield found that in a large number of the tubes of culture medium containing catgut sutures the first evidence of growth appeared as late as the twelfth or thirteenth day, and in order to provide a margin of safety, they recommended an incubation period of fifteen days.

*Neutralizing Fluids*—Various chemical substances used in the treatment of the catgut itself or in the tubing fluid, even when present in very high dilution, will often inhibit the growth of bacteria. Hence, they exert a bacteriostatic action. Unless these chemicals are dissolved and removed from the catgut, so that their action is completely neutralized, they will be carried over into the culture medium where they will inhibit growth of bacteria that may be contained within the catgut.

The first to advise the use of neutralizing fluids was Geppert<sup>6</sup> who recommended ammonium sulphide for removing mercurial salts. About six years ago Bulloch advocated the use of one per cent sodium thiosulphate as a neutralizing solution for metallic salts. Later, he and his coworkers recommended the use of ammonium sulphide, or sodium thiosulphate with sodium carbonate. The British Therapeutic Substances (Catgut) Regulations of 1930 specify one per cent sodium thiosulphate with one per cent sodium carbonate as a neutralizing solu-

tion, and this solution was adopted and recommended by Meleney and Chatfield for removing the chemicals from catgut

*Controls*—Adequate controls are essential if the bacteriologic test is to prove reliable and efficient. Meleney and Chatfield attempted to surround their standard test with every precaution, and the controls which they specified are both important and necessary. However, in addition to their controls, other controls are also required if the test is to be adequately safeguarded. In carrying out the standard test, I use the following three additional controls which I feel are essential: (1) Two or more tubes of culture medium are planted with 10 c c of each lot of distilled water and incubated for fifteen days to determine the sterility of the water. (2) At least two tubes of culture medium are planted with 10 c c of each lot of the neutralizing fluid and incubated for fifteen days to determine sterility of the solution. (3) Two tubes or more of the culture medium not planted with catgut, but sealed with a layer 2 cm. thick from every batch made of the "vaspar" mixture, are incubated fifteen days to determine sterility of the "vaspar" seal.

During the past fourteen months, I have been using the proposed standard bacteriologic test, with the additional three controls just mentioned, for determining the sterility of surgical catgut sutures. Many thousand lots of sutures have been tested during this time, so that I have had ample opportunity of determining whether or not the test is effective.

#### CONCLUSIONS

1 Adequate controls are essential for the effective application of the standard bacteriologic test, and three additional controls are herein described and recommended.

2 The standard test for sterility, proposed by Meleney and Chatfield, when used with the three additional and essential controls herein described, and when used in conjunction with suitable neutralizing fluids for dissolving and removing the chemical compound with which the sutures may be impregnated, represents the most efficient and reliable control of the sterility of surgical catgut sutures.

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## A MODIFICATION OF TECHNIC IN TERRY'S METHOD OF RAPID TISSUE DIAGNOSIS\*

H A ROTHSOCK, JR., M D , BETHELSBURG, PA

THE following technic has been found very satisfactory, particularly in the cutting of uterine scrapings for rapid diagnosis. In Terry's original technic a thin slice of the tissue for examination is cut with a razor. The surface of this tissue is then stained with a polychrome methylene blue, the excess stain is washed off and the tissue is examined. While this method can be applied to solid pieces of tissue it is not applicable to uterine scrapings and other small bits of tissue.

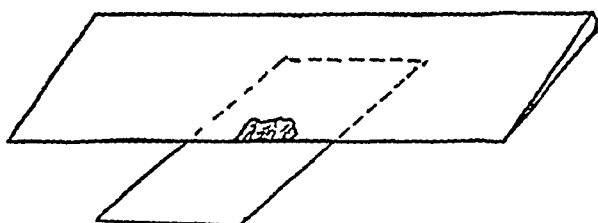


Fig 1—Position of slide in reference to knife and tissue

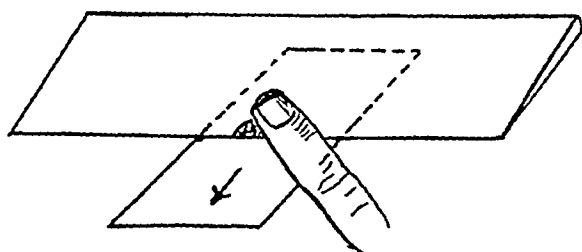


Fig 2—The finger and slide are both moved in the direction of the arrow

The difficulty of cutting small pieces of soft tissue can be overcome by first freezing the pieces and then cutting the frozen mass as follows. The uterine scrapings or other small pieces of tissue are placed on the stage of a freezing microtome and frozen en-masse. While the tissue is still frozen, a section is cut about 1/16 to 1/32 of an inch in thickness. The thick section is prevented from "rolling up" by guiding it with the index finger of the left hand. The frozen section then lies flat on the microtome knife. The frozen section is then quickly transferred to a slide before thawing takes place. This is done by placing a slide perpendicular to the cutting blade of the knife and partially under that portion of the knife on which the section lies (Fig 1). Then while the left hand slowly draws the slide forward the index finger of the right hand is placed on the partially frozen section and it is also drawn forward (Fig 2), transferring the section onto the slide. This operation must be done rapidly as it will be found

\*Received for publication February 1 1932

very difficult to keep the numerous small pieces of tissue flat and together if the thick section is not partially frozen during the transfer. The section is then stained by placing two or three drops of Terry's stain on the tissue. The slide and section are then immersed in water, the slide being held horizontal during this process and the immersion being carried out very slowly as any currents of water are liable to dislodge the pieces of tissue. When the proper amount of stain is washed off, which will be a matter of a few seconds, the slide is removed from the water, being still held in an even position to prevent the tissue from being washed off. The excess water and stain are then wiped off the slide and the cover slip is placed on the stained tissue. It is then ready for microscopic examination.

1016 DELAWARE AVENUE

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## A COLLOIDAL CARBON FLOCCULATION TEST IN SPINAL FLUID\*

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### PRELIMINARY REPORT

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ALTHOUGH there have been advocated a number of methods with varying techniques in attempting a fractional analysis of spinal fluid, all possess defects which improvement and refinement fail to remove. However, with the introduction of each of these there has been given to the field of the chemistry of spinal fluid an additive or eliminative contribution. These contributions serve two purposes: first, they add to or subtract from the diagnostic facilities of the laboratory, and second, they advance, in every instance, the understanding of the mechanisms underlying the pathology of the fluids.

It is because of these that we feel justified in presenting a preliminary report of a study in colloidal chemistry of a flocculation reaction of colloidal carbon in a series of spinal fluids. The theoretical aspects of the underlying chemical principles will be presented in a later and more extensive report of the work. Herein are tabulated the laboratory results with comparative tests which are routinely performed upon all spinal fluids in this institution.

#### METHOD OF SECURING SPINAL FLUID

All cases admitted to the Colorado Psychopathic Hospital receive admission lumbar punctures from which fluid is drawn into sterile tubes for total protein, sugar, Wassermann and colloidal gold tests. Part of this fluid was used for the colloidal carbon flocculation test. Each fluid was set up in duplicate in the later set.

The fluid for the colloidal carbon flocculation test was placed in an ice box until used. The length of time during which the fluid stood did not affect the test as long as the fluid remained sterile. All contaminated fluids were discarded.

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\*From the University of Colorado Psychopathic Hospital and Medical School.  
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## METHOD OF PREPARATION OF TEST

Seven tubes which had been washed thoroughly rinsed with distilled water and dried were used. For convenience test tubes of 100 mm by 13 mm and of 125 mm by 16 mm were used. The size or caliber of the tube used did not affect the test. These were placed in a rack and numbered 1, 2, 3 etc. through 7.

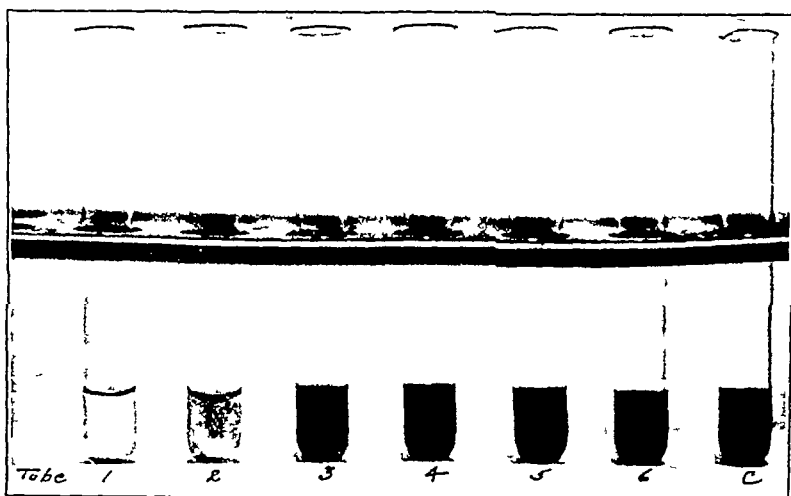


Fig 1

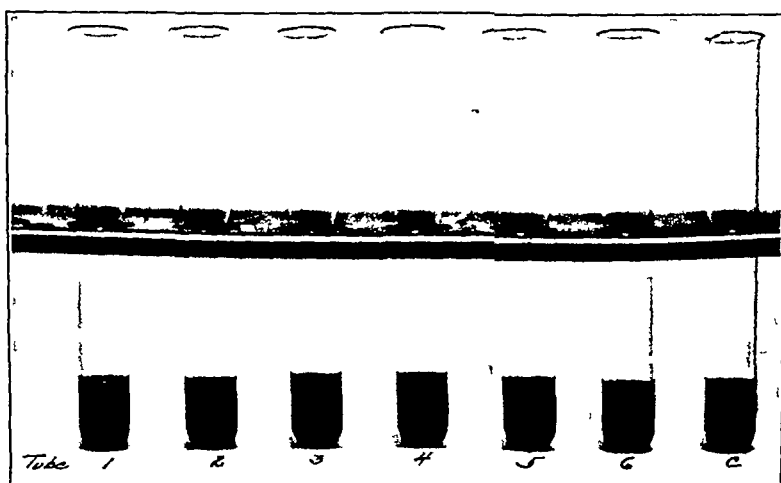


Fig 2

In each tube was placed 1 cc of distilled water. In tube No 1 was placed 1 cc of spinal fluid. The contents of tube No 1 were thoroughly mixed and 1 cc of this mixture was transferred to tube No 2. This process was repeated through tube No 6, the last 1 cc being discarded. Tube No 7 acted as the control.

In each of the seven tubes was then placed 0.1 cc of 0.1 per cent ovalic acid solution. Each tube was again thoroughly mixed. In each tube was

then added 0.4 c.c. of 1 per cent colloidal carbon (The 1 per cent colloidal carbon was made by diluting 1 c.c. of Carter's Black India Ink No 358 to a total volume of 100 c.c. with distilled water at room temperature) Each tube was finally agitated until thoroughly mixed

The tubes were then set aside at room temperature and read twelve hours later

#### METHOD OF READING TUBES

The tubes were read, dependent upon the result obtained, as either "flocculation" or "no flocculation" The "flocculation" was a definite aggregation of the colloidal carbon particles which settled to the bottom of the tube leaving a clear, supernatant fluid The "no flocculation" was a stable suspension of the colloidal carbon particles and tube No 7 served as this control Any questionable degree of murkiness between these two extremes was considered as "no flocculation"

Fig 1 is a typical "flocculation" set Fig 2 is a typical "no flocculation" set

The "flocculation" was designated by the letter "P" The "no flocculation" was recorded as "O"

#### RESULTS

Tables I and II, are the findings in the first 100 cases upon which the test was used

TABLE I

DIAGNOSIS	NUMBER OF CASES
General paresis	26
Schizophrenia (Dementia precox)	20
Senile psychosis	7
Organic brain disease	6
Epilepsy (idiopathic)	6
Alcoholism	6
Manic depressive psychosis, depressed phase	5
Cerebro arteriosclerosis	3
No psychosis	2
Manic depressive psychosis, manic phase	2
Taboparesis	2
Meningovascular syphilis	2
Psychoneurosis	2
Posttraumatic constitution	2
Manic depressive psychosis	1
Hydrocephalus	1
Diabetes insipidus	1
Multiple sclerosis	1
Cerebral hemorrhage	1
Constitutional psychopathic inferiority	1
Mental defective	1
Toxic psychosis	1
Korsakow's psychosis	1
Total	100

#### SUMMARY

- 1 The method of conducting a colloidal carbon flocculation test in spinal fluid is described
- 2 The results obtained with this test in 100 consecutive cases are presented

TABLE II

CASE NUMBER	DIAGNOSIS	AGE	H OOD WASSERMANN	SPINAL FLUID WASSERMANN	SPINAL FLUID ROBIN	SPINAL FLUID SUGAR	GOLD CURVE	COLLOIDAL CURVE	TREATMENT	PREVIOUS WORK			
										H OOD WASSERMANN	SPINAL FLUID WASSERMANN	GOLD CURVE	
1	General Paresis	55	0 0	4 1	130	39	5555544320	PP000000					
2	General Paresis	52	0 0	0 0	10	50	1113310000	P0000000	T	0 2	4-4	3334555540	
3	General Paresis	38	4 4	4 4	130	53	5555544430	PPP00000					
4	General Paresis	38	4 4	4 4	35	62	5555410000	PP000000					
5	General Paresis	47	0 0	4 4	20	50	5554432000	PP000000					
6	General Paresis	56	0 0	0 0	25	72	0000000000	P0000000	T	0 0	4 4	4444555100	
7	General Paresis	38	4 4	0 0	18	77	0112210000	PP000000					
8	General Paresis	20	0 0	0 0	50	80	2223321000	PP000000					
9	General Paresis	45	4 4	4 4	70	37	5555443100	PP000000					
10	General Paresis	39	0 2	4 4	40	90	5554443200	PP000000					
11	General Paresis	44	0 0	3 4	30	54	2223200000	PP000000	T	0 0	3-4	2223200000	
12	General Paresis	41	0 0	0 0	70	53	2223321000	P0000000	T	4-4	4-4	3333455500	
13	General Paresis	39	0 0	0 0	38	103	5544310000	P0000000	T	0 0	1 4	1111220000	
14	General Paresis	35	0 0	1 4	30	74	2223443000	P0000000	T	3 3	4 4	2233455440	
15	General Paresis	42	4 4	0 0	15	78	0000000000	PP000000	T	—	4 4	1555442100	
16	General Paresis	45	0 0	0 0	55	81	0001110000	P0000000	T	—	0 4	4455555550	
17	General Paresis	31	4 4	4-4	50	81	5554321000	PP000000	T	4-4	4-4	5555421000	
18	General Paresis	36	—	3 2	20	58	5555444310	PP000000	T	—	4 4	5555431000	
19	General Paresis	40	—	0 0	28	50	0011310000	P0000000	T	0 0	—	0112210000	
20	General Paresis	58	—	0 0	33	72	0000000000	PP000000	T	4-4	4-4	3344445550	
21	General Paresis	51	—	4 4	75	66	5555443210	PP000000	T	0 0	4-4	5544322000	
22	General Paresis	33	2 4	4 4	80	64	5555554440	PP000000					
23	General Paresis	59	4 4	4 4	105	77	5555544420	PP000000					
24	General Paresis	60	0 4	4 4	155	50	5555543320	PP000000					
25	General Paresis	45	—	4 4	70	53	5555543200	PP000000					
26	General Paresis	19	4 4	4 4	100	72	5555544430	PP000000					
27	Tubo Paresis	52	4 4	4 4	60	98	0011222100	PP000000					
28	Tubo Paresis	54	0 0	0 0	70	86	0011210000	P0000000	T	—	3 4	2112100000	
29	Meningo Vascular Syphilis	26	—	4 4	75	69	5555544110	PP000000					
30	Meningo Vascular Syphilis	27	0 0	4 4	40	74	2223321000	PP000000					
31	Multiple Sclerosis	35	0 0	0 0	40	76	5555544110	00000000					
32	Schizophrenia	44	0 0	0 0	55	75	0000000000	00000000					
33	Schizophrenia	26	0 0	0 0	23	48	0000000000	00000000					
34	Schizophrenia	35	0 0	0 0	23	90	0000000000	00000000					
35	Schizophrenia	25	0 0	0 0	60	91	0011100000	00000000					
36	Schizophrenia	40	0 0	0 0	15	58	0011000000	00000000					
37	Schizophrenia	38	0 0	0 0	20	66	0000000000	00000000					
38	Schizophrenia	44	0 0	0 0	20	50	0000000000	00000000					
39	Schizophrenia	53	0 0	0 0	50	85	0001100000	00000000					
40	Schizophrenia	40	0 0	0 0	35	81	0000000000	00000000					
41	Schizophrenia	52	0 0	0 0	33	90	0000000000	00000000					
42	Schizophrenia	19	0 0	0 0	55	68	0000000000	00000000					
43	Schizophrenia	42	0 0	0 0	43	55	0001100000	00000000					
44	Schizophrenia	39	0 0	0 0	25	90	0000000000	00000000					
45	Schizophrenia	28	0 0	0 0	30	94	0000000000	00000000					
46	Schizophrenia	49	0 0	0 0	55	83	0000111000	00000000					
47	Schizophrenia	22	0 0	0 0	33	85	0000000000	00000000					
48	Schizophrenia	20	0 0	0 0	50	66	0001110000	00000000					
49	Schizophrenia	30	0 0	0 0	35	67	0012100000	00000000					
50	Schizophrenia	20	0 0	0 0	15	60	0000000000	00000000					
51	Schizophrenia	25	0 0	0 0	60	91	0001110000	00000000					
52	Senile Psychosis	55	0 0	0 0	30	40	0000000000	00000000					
53	Senile Psychosis	86	0 0	0 0	32	73	0001110000	00000000					
54	Senile Psychosis	66	0 0	0 0	58	92	0000000000	00000000					
55	Senile Psychosis	59	0 0	0 0	73	69	0001110000	00000000					
56	Senile Depression	45	0 0	0 0	20	69	0000000000	00000000					

TABLE II (CONTINUED)

CASE NUMBER	DIAGNOSIS	AGE	BLOOD WASSERMANN	SPINAL FLUID WASSERMANN	SPINAL FLUID PROTEIN	SPINAL FLUID SUGAR	GOLD CURVE	COLLOIDAL CURVE	TREATMENT	PREVIOUS WORK			
										BLOOD WASSERMANN	SPINAL FLUID WASSERMANN	GOLD CURVE	
57	Senile Deterioration	69	0 0	0 0	50	75	0000000000	00000000					
58	Senile Psychosis	64	0 0	0 0	43	82	0012200000	00000000					
59	Organic Brain Disease	35	0 0	0 0	50	72	0000000000	00000000					
60	Organic Brain Disease	41	0 0	0 0	60	27	0000111000	00000000					
61	Organic Brain Disease	27	0 0	0 0	28	61	0000000000	00000000					
62	Organic Brain Disease	52	0 0	0 0	45	104	1112100000	00000000					
63	Organic Brain Disease	48	0 0	0 0	70	63	0001110000	00000000					
64	Organic Brain Disease	45	1 3	0 0	35	70	0001110000	00000000					
65	Epilepsy	23	0 0	0 0	15	81	0000000000	00000000					
66	Epilepsy	13	0 0	0 0	20	70	0011100000	00000000					
67	Epilepsy	51	0 0	0 0	45	70	0012100000	00000000					
68	Epilepsy	11	0 0	0 0	35	65	0000000000	00000000					
69	Epilepsy	2	0 0	0 0	35	72	0000000000	00000000					
70	Epilepsy	17	0 0	0 0	43	66	0001100000	00000000					
71	Alcoholism	36	0 0	0 0	40	96	0000000000	00000000					
72	Alcoholism	40	0 0	0 0	15	75	0000000000	00000000					
73	Alcoholism	41	0 0	0 0	25	93	0000000000	00000000					
74	Alcoholism	24	0 0	0 0	15	64	0000000000	00000000					
75	Alcoholism	38	0 0	0 0	43	58	0000000000	00000000					
76	Alcoholism	34	0 0	0 0	25	—	—	00000000					
77	Depression	33	0 0	0 0	18	59	0001110000	00000000					
78	Depression	20	0 0	0 0	25	57	0000000000	00000000					
79	Depression	37	0 0	0 0	60	69	0000000000	00000000					
80	Depression	41	0 0	0 0	25	96	0000000000	00000000					
81	Depression	53	4 4	0 0	20	75	0000000000	00000000					
82	Arteriosclerosis	68	0 0	0 0	25	50	0000000000	00000000					
83	Arteriosclerosis	82	0 0	0 0	28	98	0000000000	00000000					
84	Arteriosclerosis	62	0 0	0 0	20	80	0000000000	00000000					
85	No psychosis	45	0 0	0 0	40	85	0000000000	00000000					
86	No psychosis	39	0 0	0 0	15	73	0000000000	00000000					
87	Manic with Paranoid Trends	47	0 0	0 0	50	72	0000000000	00000000					
88	Manic Psychosis	24	0 0	0 0	28	72	0000100000	00000000					
89	Psychoneurosis	21	0 0	0 0	25	63	0000000000	00000000					
90	Psychoneurosis	45	0 0	0 0	70	117	0001110000	00000000					
91	Post Traumatic Constitution	36	0 0	0 0	28	95	0001100000	00000000					
92	Post Traumatic Epilepsy	31	0 0	0 0	28	58	0011100000	00000000					
93	Mental Defective	28	0 0	0 0	70	74	0000000000	00000000					
94	Cerebral Hemor- rhage	35	0 0	0 0	28	67	0000000000	00000000					
95	Toxic Psychosis	34	0 0	0 0	15	91	0001100000	00000000					
96	Manic Depressive Psychosis	29	0 0	0 0	30	77	0000000000	00000000					
97	Diabetes Insipidus	14	0 0	0 0	25	54	0001100000	00000000					
98	Hydrocephalus	2	0 0	0 0	66	25	0011000000	00000000					
99	Psychopathic Inferiority	28	0 0	0 0	20	121	0000000000	00000000					
100	Korsakoff's Psychosis	41	0 0	0 0	25	96	0000000000	00000000					

with the comparative laboratory work—gold curve, Wassermann (blood and spinal fluid), spinal fluid protein and spinal fluid sugar

3 There appears to be a definite correlation between this test and neurosyphilis

The authors wish to express their appreciation for the constructive criticism of the work by Drs George S Johnson, Edward R Mudge, and Robert C Lewis

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## AN AUTOMATIC PIPETTE FOR THE ACCURATE DELIVERY OF VARIABLE QUANTITIES OF LIQUIDS\*

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PHILIP L VARNEY, M S, AND DONALD M HETTER, PH D ST LOUIS, MO

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VARIOUS laboratory procedures, such as the examination of water, milk, and sewage for total bacterial count, require the use of dilution flasks containing exact quantities of sterile water. Certain other procedures require the use of large numbers of tubes containing known quantities of culture media. Since the distribution by hand of these liquids is very time consuming several pipettes have been designed to automatically measure and distribute this material. In general, these appliances which are usually made in the form of a four-way stopcock so connected to two side arms that one of these arms is filling while the other is emptying have a serious disadvantage in that they deliver only a fixed quantity of fluid and any losses in the amounts delivered, due to subsequent sterilization can be remedied only by the direct addition with a pipette of fresh liquid to the individual containers. Furthermore, since these devices are constructed of glass, they are easily broken necessitating care in their handling.

Since the amount of liquid lost from a container during the process of sterilization varies with the composition, size, and shape of the container, the amount and nature of the fluid contained therein, and the peculiarities of the particular autoclave in use, the use of a delivery pipette of fixed capacity for the distribution of liquids is often very undesirable. To be satisfactory for general laboratory use, an automatic pipette should not only be unbreakable, but should be so constructed that it can be rapidly and easily adjusted to any desired capacity.

Requiring in our laboratory numerous tubes of culture media the volume of which after sterilization had to be kept within extremely close limits, we endeavored to design a delivery device so constructed as to overcome the disadvantages inherent in existing types of automatic delivery pipettes. The details of construction of this apparatus, which has been designed throughout with the object of speed and accuracy in use, are given below †

The outer section or shell of the apparatus consists of a four-way stopcock, constructed of brass or bronze stock, from the two sides and front of which project side arms, as shown in Figs 1, 2, and 3. All external parts of the apparatus

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†This apparatus may be obtained from the Arthur H Thomas Company Philadelphia

are thoroughly polished so as to aid in cleaning the device. For the sake of strength and appearance, the top of the shell terminates in a heavy shoulder.

A somewhat smaller side arm projects from the rear of the shell to which is fastened an inlet tube for the liquid being distributed. Should it prove difficult to make the apparatus in one piece similar to the manufactured article, the side arms may be separately turned from brass or bronze stock, and fastened to the main shell by means of properly made soldered joints.

With the aid of a lathe, the shell is carefully hollowed out, leaving an opening extending completely through the apparatus. The walls of this opening converge inward from top to bottom at an angle of about  $32^\circ$ , and must be carefully finished to fit the inner, movable portion of the stopcock, to prevent the accidental

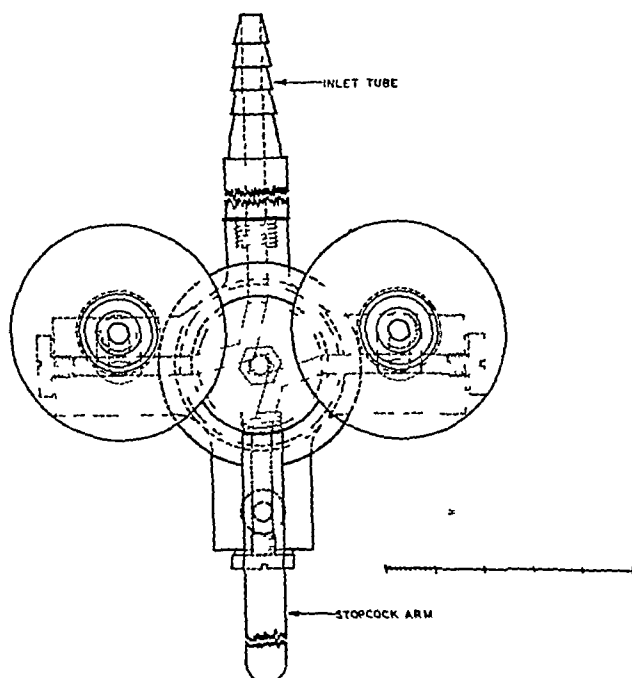


Fig. 1—Top view of automatic test tube filler. (Main divisions of scale indicate centimeters.)

escape of liquid from the device. The shell walls should be 3 to 4 mm in thickness.

The inner movable portion of the stopcock is constructed of brass or bronze stock, carefully machined so that when ground into position, the top projects approximately 12 mm above the shoulder of the outer shell when placed in position, as shown in Fig. 2. A handle of any desirable length and diameter is fitted into the projecting portion of this center piece by means of a threaded or soldered joint.

Holes 4 mm in diameter are drilled through the front, back and side arms of the outer shell at angles exactly  $90^\circ$  to each other as shown in Fig. 1. The movable portion of the stopcock is then drilled as shown in Fig. 1 so that the various openings exactly coincide with the holes in the outer shell. The holes

must accurately coincide whether the movable arm is turned straight forward, or  $90^\circ$  to either side of the forward position

The inlet tube, which is made so as to tightly hold a rubber tube, is fastened to the rear arm of the shell by means of threaded connections. The bore of this tube should be 4 mm in diameter.

An outlet tube, the bore of which is 4 mm. in diameter, is connected to the front arm of the shell by means of a soldered joint. The tip of the outlet tube is sharply bevelled, to insure accuracy of delivery of the fluid.

Two side arm uprights, made of heavy wall brass tubing of 4 mm internal diameter, are constructed as shown in Figs 2 and 3. These uprights are coarsely threaded for all but a short portion of their lower length so that movable brass

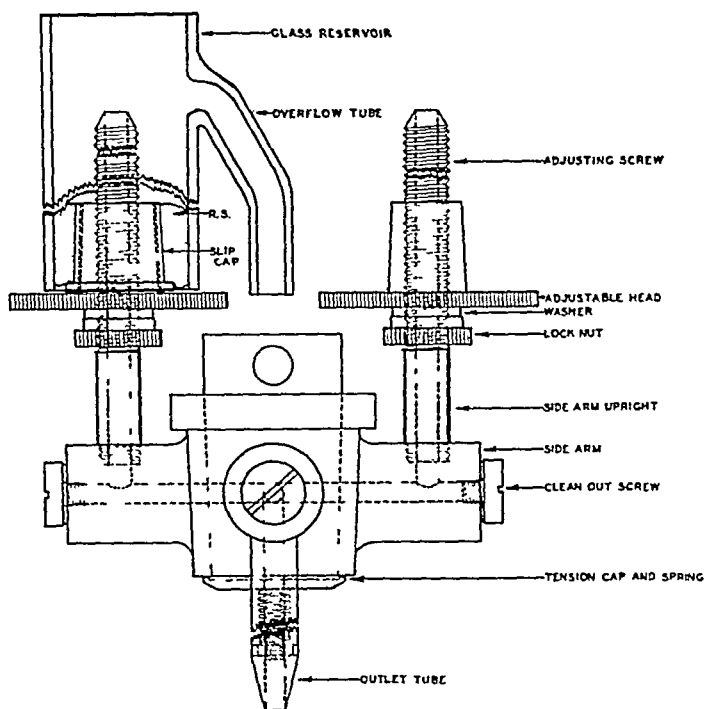


Fig 2—Top view of test tube filler showing one glass reservoir in place and the method of attaching the reservoirs to the adjustable heads

platforms, tapped to fit the threads of the tubing, can be adjusted to occupy any position on the tubes. These adjustable heads or movable platforms can be locked in position at any point by means of leather washers and brass lock nuts as shown in the diagrams. The threaded portions of the tubes should be of such a length that the adjustable heads have a range of motion of at least 9 to 10 cm. The upper ends of the uprights are carefully bevelled so as to produce sharply defined menisci, thus increasing the accuracy of volume of the various portions of liquid withdrawn from the outlet tube.

The adjustable heads consist of large flat platforms, the upper sides of which terminate in cones machined so that tapered brass shells or caps can be placed in position over them and seated by means of a twisting motion so as to produce a

water tight seal. These caps should be fitted so as to rotate freely without binding.

Glass reservoir tubes of various capacities are made, to be attached to the tapered slip caps by means of rubber or wax seals. Each glass reservoir has an overflow tube so located that, with the adjustable head about 2 cm. from its highest possible position the maximum desired volume of liquid is retained within the reservoir when the stopcock is closed. The additional 2 cm. of threading permits further increasing the capacity of the reservoirs to compensate for any losses due to evaporation during sterilization. The capacity of the reservoirs can be adjusted within rather wide limits for larger or smaller amounts of fluid by means of the adjustable heads.

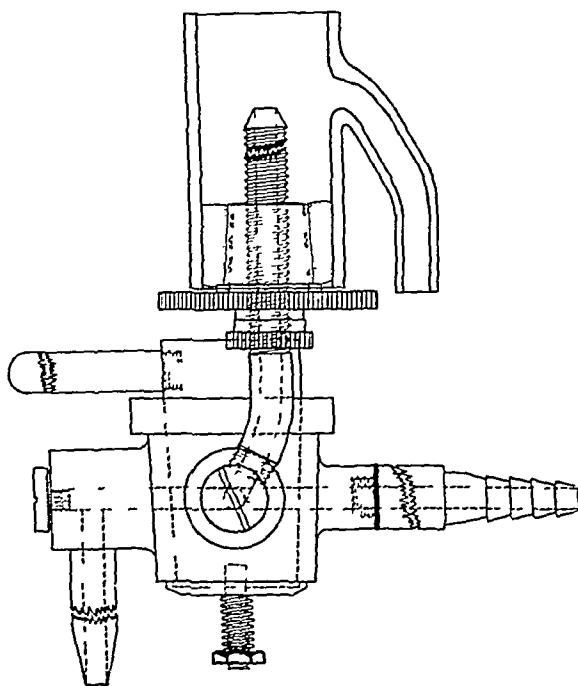


Fig. 3.—Side view of test tube filler showing details of construction of the spring tension device.

When the apparatus is in use, the two glass overflow tubes are connected together by means of a Y joint, and the excess liquid collected in a container placed at the rear of the apparatus.

The two uprights are fastened to the side arms by means of tightly fitting threaded joints. The side arms are drilled and tapped so that when the upright tubes are fitted and before they are bent into their final position, they point slightly to the rear, rather than straight up and down. Then, with the stopcock arm turned 90° either to the left or the right hand side position, so that the various holes are in accurate alignment, the uprights are bent as shown in Fig. 3 until their outer walls just touch the movable arm thus acting as stops for the latter.

The opening in the ends of the side and front arms are closed by means of



screws and washers. The apparatus can be thoroughly cleaned by removing these screws and the center movable arm.

Constant and even contact between the center and outer portions of the stop cock is assured by means of a spring tension device, the details of which are shown in Fig. 3.

Rapid and very accurate delivery of any desired amount of liquid into test tubes or flasks is possible by the use of this apparatus. Various sized reservoirs can be used interchangeably, so that by their use it is possible to secure any volume of delivery merely by varying the height of the reservoirs with the aid of the adjustable heads.

## A SIMPLE PHOTOGRAPHIC KYMOGRAPH AND TIME RECORDING METHOD\*

E. W. GRAY, M.D., THILLIS, N. Y.

**M**ETHODS of employing the smoked paper kymograph for recording various physiologic processes are quite cumbersome, especially where records are to be made on a fairly rapidly moving drum. A few of the difficulties may be summed up briefly as follows:

The writing points are frequently either too tight and refuse to move or are too loose and do not write at all. It is quite necessary that the surface of the kymograph drum and the arc of movement of the writing levers be parallel, otherwise the levers either leave the surface or become too tight when they move. Another disadvantage is the lag caused by friction between the points and the smoked paper. It is scarcely necessary to mention the untidiness brought about by the use of a shellac fixing solution and by smoking the paper.

The use of inked writing points has some of the above disadvantages, and in addition, the ink sometimes stops flowing in the middle of an experiment. The photographic method of recording various physiologic processes is by no means new. The electrocardiograph and photographic polygraph have fully demonstrated this principle. Such apparatus is, however, very expensive.

In order to overcome the latter difficulty, we constructed a simple photographic casing in our laboratory to fit a standard make kymograph. This consists of a square box split vertically in the center to fit around the kymograph drum. The box is constructed of wood except for the front which is a strong sheet of metal with a central vertical slit which when in place lies very close to the surface of the drum. A sliding shutter closes the slit to light and is held in place by a trigger. The shutter (see Fig. 1) opens automatically by the tripping of the trigger when a side arm (see Fig. 2) attached to the shaft of the kymograph strikes one arm of a forked lever. After one complete revolution the side arm strikes the second arm of forked lever (see Fig. 3) and releasing the opening spring allows a weaker (closing) spring to close the shut-

\*From the Research Department, Letchworth Village, New York State School for Feeble-minded.

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ter In this manner the shutter can be regulated to avoid double exposure of the sensitive paper or film

Various timing devices may be used depending on the speed and accuracy desired A timing method for a slowly moving drum has been described by Chillingworth<sup>1</sup> in which the shaft of a synchronous electric clock made a contact once a second thereby energizing a signal magnet We are employing, in reac-

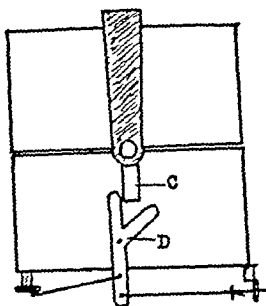


FIG 2 -  
C Side arm  
D Forked lever

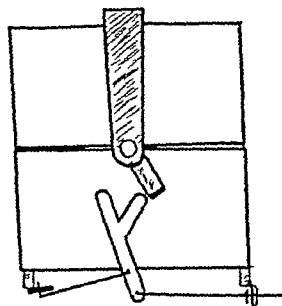


FIG 3

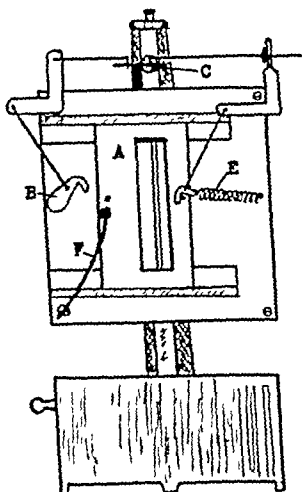


FIG 1 -  
A Shutter  
B Trigger  
C Side arm  
E Opening spring  
F Closing spring

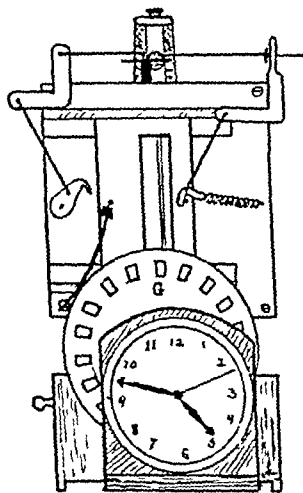


FIG 4 -  
G Timing disc

tion time experiments an aluminum disc with perforations near the edge The disc is turned by means of a synchronous electric clock exactly one revolution a second The number of perforations in the circumference determines the number of light flashes that pass through the lower end of the slit per second The disc we use in most of our work has twenty perforations and thus records intervals of a twentieth of a second

A strong beam of light is thrown through the slit. This is accomplished by means of a parallel ray electric light or sunlight reflected from a mirror. Sunlight gives the best records from a photographic viewpoint. We use rapid black bromide paper which comes in rolls twenty inches in width and can be cut as desired. A record taken in our laboratory is reproduced.

The instrument is quite applicable to such experiments as latent period of muscle, velocity of nerve impulse, reflex time, reaction time and, in fact, any experiment where a short paper kymograph is used.

We are employing the above described apparatus to determine simple reaction time to light in a series of mentally deficient children. The reaction time is recorded by means of two signal magnets placed in front of the slit. The lower signal magnet records on the paper the exact moment that a small 3-volt

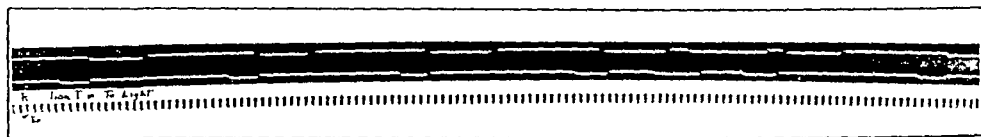


Fig 5 —Reaction time record reduced to one-third actual size

light bulb is illuminated. The subject responds by the touch of a light key which activates the upper signal magnet. In this manner not only is the time interval between stimulus and reaction recorded but the duration of the stimulus and the response and also the time interval between stimuli. Another advantage in closing the circuit in both signals is that the lag of each magnet will be practically the same.

#### SUMMARY

1 A very inexpensive photographic attachment has been developed for a standard make kymograph.

2 A simple and accurate method is described for recording short time intervals photographically.

3 A reduced photograph is shown of a reaction time record made with the apparatus described.

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## A SIMPLE APPARATUS FOR REMOVING GASTRIC CONTENTS FROM DOGS\*

S L MOSKOWITZ, B S, M S, AND C M WILHELMJ, M S, M D,  
OMAHA, NEBR

IN ORDER to obtain samples of gastric juice from dogs, we have devised a rather simple apparatus, which, in our hands, has yielded very satisfactory results. Briefly, the complete outfit consists of a box to hold the dog and a suction apparatus connected to a stomach tube to obtain the gastric samples.

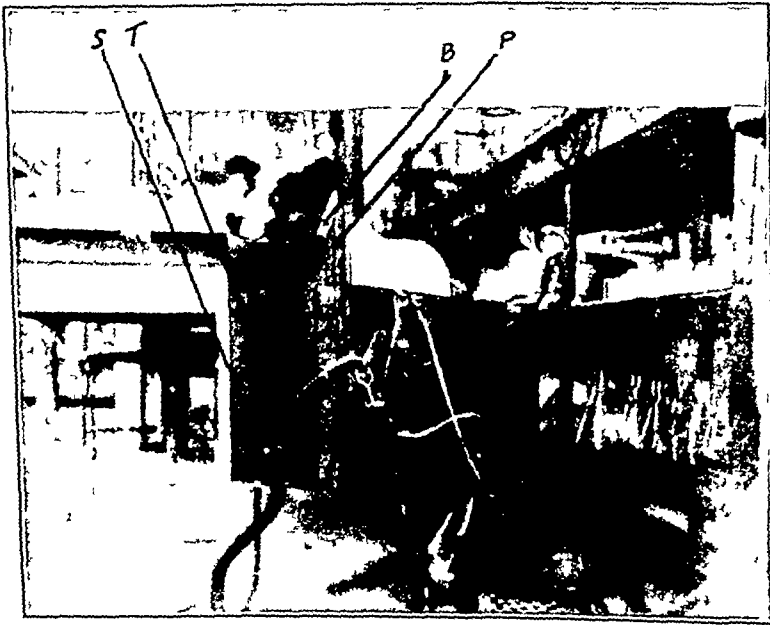


Fig 1.—B Chain ensheathed in rubber tubing around dog's neck. P Post in which front partition is inserted. S Spindle which holds front partition at desired height. T, Front partition which slides in grooves in P.

The box is so arranged that it can be adjusted to the length and height of the animal used by means of two sliding partitions, one in front sliding up and down, having a U-shaped section cut out at the top (Fig 1) in order to accommodate the neck of the dog and the other in back (Fig 2) which can be pushed forward or backward to accommodate the length of the animal. Attached to the front partition is a chain ensheathed in a small piece of rubber tubing (B, Fig 1), which fits over the dog's neck and thus prevents him from slipping away from the experimenter.

A bit, consisting of a piece of soft wood about six inches long an inch and a half wide and three quarters of an inch thick with a hole sufficiently large to

\*From the Department of Physiology, Creighton University School of Medicine.  
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allow easy entrance of the stomach tube is placed in the animal's mouth when ready for pumping

The box is thirty inches long, fourteen inches high and seven inches wide and is mounted on four legs. At intervals along the sides slits are drilled through which straps can be passed in order to secure the animal and prevent any undesirable movements (*I*, Fig 2). These straps can be passed both under and over the animal. We have never found it necessary to strap in our animals, the neck strap and the narrowness of the box being sufficient to prevent any serious disturbances at any time.

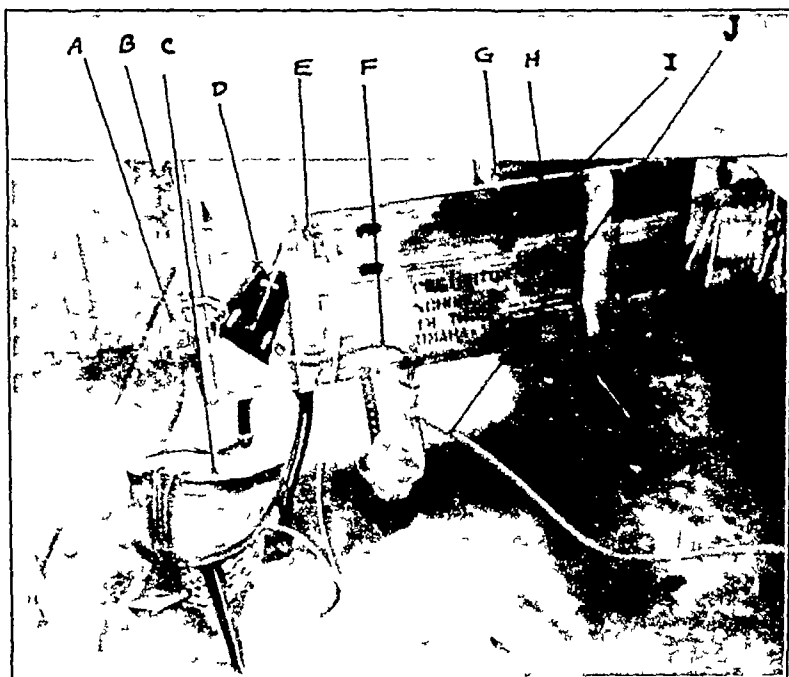


Fig 2—A Stomach tube attached to one end of T-shaped glass tubing B Same as in Fig 1 C Bellows D Wood block in which T-shaped glass tubing is sunken for protection against injury and to render secure E Two-way stopcock F Heavy rubber tubing connecting lower end of T shaped glass tubing to suction flask G Rear partition which slides forward or backward H Grooves which fix rear partition at desired place I Slits through which straps may be passed to keep animal steady J Heavy rubber tubing connecting suction flask to one of the glass tubes in rubber cork of five-gallon bottle

Along the side of the box a T-shaped piece of glass tubing is sunk in wooden grooves (*D*, Fig 2) to prevent its accidental breakage. This glass tubing is the essential feature of the apparatus.

As can be seen from Fig 2, one end of this T-shaped glass tubing is connected to a pair of bellows through a two-way stopcock (*E*). The other two ends of the T-shaped glass tubing are connected to a suction flask and to the stomach tube respectively. The suction flask is in turn connected by means of heavy rubber tubing (*J*, Fig 2) to one of two pieces of glass tubing in the rubber cork of a five gallon bottle (not shown in the figures). The other piece of glass tubing in this rubber cork is connected through heavy rubber tubing to the water suction pump. The five gallon bottle is put between the suction pump and the suction flask to prevent the contents of the suction flask from being

pulled into the water pump when the suction is turned off. It has been found advisable to partly fill the bottle with water before using.

When running an experiment the animal is placed in the box, the rear partition adjusted to his length and the front to his height, his neck is pulled forward, the chain passed over it and attached to a hook on the front partition. His mouth is opened and the bit placed transversely across his teeth. The stomach tube is then dipped into water to lubricate it so that it passes easily down the esophagus, inserted into the hole in the bit and passed down into the stomach. Suction is then turned on and within a few seconds the stomach contents will pass into the suction flask. Very often the suction causes the tube to adhere to the stomach mucosa or the tube becomes plugged with mucus or food particles. At this point we resort to the bellows. The stopcock (*E*, Fig 2) is turned so that air can be passed into the stomach, and the rubber tube connecting the side arm of the glass T-tube with the suction flask is compressed to prevent the air from getting into the suction flask and facilitate its passage into the stomach. When the stomach tube can be freed from the mucosa (determined by attempting to move it about), the stopcock *E* is turned, cutting off the bellows, the rubber tubing *F* is released and the suction is again free to act.

If fluids are put into the stomach by means of a stomach tube an almost quantitative return can be obtained in a few seconds.

We have found that after the first few experiments our dogs rapidly become accustomed to the sensation of a tube being passed and in a very short time offer no resistance.

## AN IMPROVED MARKING FOR PRECISION SYRINGES

WARREN T. VAUGHAN, M.D., RICHMOND, VA

THE familiar so-called tuberculin syringe has found probably its greatest utilization in allergic diagnosis and therapy. The allergist very often has occasion to recommend certain dosages of treatment extracts which are to be given either by the family physician or by the patient himself. The presence on all hypodermic syringes of two different scales, the metric and the minim, frequently leads to confusion. No matter how careful one may be in stressing the fact that dosages should be given on the basis of the metric scale, too often the physician or the patient or the nurse uses the minim scale, thereby giving the incorrect dose.

As an example, a patient was being treated by his family physician and was supposed to be getting 0.6 cc of 2 per cent ragweed extract. Both the patient and the physician assured me that this was what he was receiving. Reporting to the office for one of his treatments he was given 0.6 cc of 2 per cent ragweed extract which was promptly followed by a mild general anaphylactic reaction. Investigation in collaboration with the physician then developed that after all the patient had been receiving 6 minims instead of 0.6 cc. When he did receive 0.6 cc he was therefore given a 50 per cent larger dose.

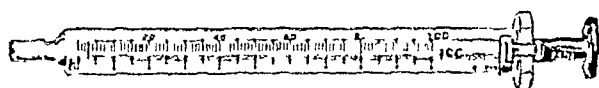
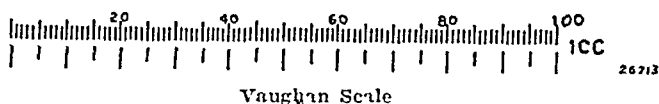
The administration of insulin, tuberculin, vaccines, particularly arthritis vaccines, and all other substances in which minute, carefully measured doses must

be given is fraught with this same potential source of inaccuracy as long as there are two different scales on the syringe and when more than one person may be administering the treatment

To circumvent this disadvantage Becton Dickinson and Company have collaborated with me in the manufacture of a precision syringe of the tuberculin syringe type, possessing the following advantages

1 Only the metric scale is used, with graduation to hundredths of a cubic centimeter

2 In place of the minim scale there is a coarser graduation in tenths and twentieths of a cubic centimeter



Vaughan Syringe

3 The white line running the length of the barrel, between the two scales is omitted, thus providing an observation channel through which the position of the tip of the plunger may be observed

4 The etching of the graduations on the ordinary tuberculin syringe, while narrow, are still wide enough to cause some small variation in the accuracy of the dosage. The width of the etching on the new syringe, being only five-eighths that of the old, increases precision

5 In the old style tuberculin syringe when the plunger is pushed way in, its tip does not always correspond exactly to the zero on the scale. In this case, one must be careful in giving hypodermics not to drive the plunger home but to press it just to the zero mark. The new syringe has the zero graduation as accurately as possible on a level with the tip of the plunger when fully inserted

# NOTE ON A METHOD FOR THE MANIPULATION OF BLOOD-DILUTION AND MICRO-PIPETTES\*

PAUL L. KIRK, PH D, AND RODERICK CRAIG, PH D, BERKELEY, CALIF

IT IS a well known fact that the manipulation of pipettes for dilution of blood for corpuscle counts is both awkward and liable to be inaccurate due to the difficulty of setting the meniscus accurately on the calibration marks. Inaccuracies of several percentage may be introduced from this source alone unless great care is exercised, and if this is done, the loss of time is considerable. For these reasons, we have constructed the blood-dilution pipette shown in Fig 1, A. The diagram illustrates the design when a dilution of 1 to 10 is desired as in counting the white cells. A sort of syringe was constructed by grinding a plunger into a tube of 5 mm. inside diameter, and this was sealed to the top of a blood-dilution pipette. The device is operated entirely with one hand, thus making it entirely practical for the medical profession, since the ear or finger from which the blood is taken is normally held with the other. The top of the plunger is readily handled with the thumb and forefinger, while the body of the pipette is grasped between the other fingers and the palm of the hand. The plunger is lubricated with a little oil or vaseline. With this device, it is a very simple matter to accurately and rapidly set the meniscus exactly on the calibration mark without any secondary adjustment whatever. Moreover, the use of a mouthpiece is entirely eliminated.

In microanalysis, the Pregl<sup>1</sup> type of pipette is habitually used. Experience with this type of pipette has shown that two difficulties are encountered. The first is the disadvantage encountered with blood-dilution pipettes, viz, that the meniscus is difficult to adjust accurately since the zero mark is placed on a greatly constricted portion of the tube. The second lies in the fact that if, as is usually done, the pipette is sucked full to a point above the mark, and allowed to drop to the mark, the tube is wet above the mark, and on the subsequent washing out, this liquid is also washed out. While this is a small error, it prevents the highest precision from being attained.

At first we intended to make a series of pipettes equipped with syringes as an integral portion of the apparatus, as was done with the blood dilution pipette. Since our needs were for a graded series of pipettes having a range of volumes from about 0.01 cc to 0.1 cc and a considerable number were desired, it would have been very laborious to construct all these as planned. We have instead made the pipette series as illustrated in Fig 1, B, from capillary tubing, and in the top of each we have fitted a needle from a tuberculin syringe of 0.5 cc capacity, and sealed it in place with cement. The tuberculin syringe itself can now be used with an indefinite number of pipettes, and the difficulties above enumerated are entirely overcome. Pipettes are easily constructed in such a manner as to have any desired volume and almost any desired accuracy. The

\*From the Division of Biochemistry, University of California Medical School.  
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capillary is somewhat constricted at the point where the calibration mark is placed, so that the unavoidable error of reading may be reduced to almost any desired value. Too great constriction gives rise to difficulties due to capillarity. If small enough capillary tubing is available, this constriction is unnecessary. An error of 0.1 mm in reading the meniscus causes an error of 0.02 c mm if the bore is 0.5 mm in diameter. The pipette must be calibrated with mercury, and washed out in use as is done with the Piegel type of pipette. It must be mentioned that the same syringe and general construction are equally applicable to much larger pipettes than those built by us.

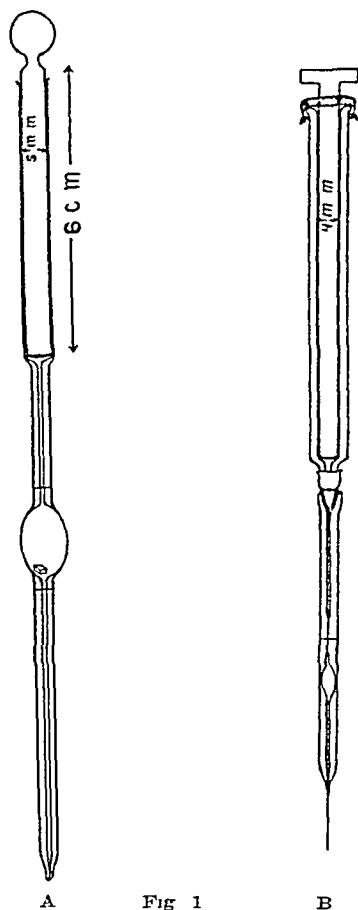


Fig. 1

A similar method of manipulating pipettes has been suggested by Duft.<sup>2</sup> These were of the ordinary macro variety, where fewer advantages of speed and accuracy are gained than with those described by us. Moreover, his arrangement was adapted to use with bacterial cultures, largely for the sake of safety rather than to achieve extraordinarily fine manipulative technique. The rubber connections used in that design would make it next to impossible to calibrate a pipette with mercury, as we have found from experience, since the great suction exerted by a column of mercury distorts the rubber connection, and makes an accurate adjustment of the meniscus very difficult. Both the uses and design of

our apparatus are different, and we believe the advantages to be gained here are even greater than those gained by Duff in the solution of his particular technical difficulties

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- 2 Duff, D C B Improved Pipette Manipulator, J LAB & CLIN MED 15 1027, 1930

### NOTES ON APPARATUS FOR PERFORMING THE KLINE TEST\*

THOMAS B MAGATH, M D , ROCHESTER, MINN

THE increasing popularity of the Kline test and its use in large institutions has made it desirable to develop certain apparatus to facilitate the performance of the test

#### PIPETTES FOR ANTIGEN

The pipette for the antigen, as recommended by Kline, is made by pulling out a piece of glass tubing so that the tip has an aperture which will permit a drop of antigen of from 0.0075 to 0.0085 cc to be delivered. This pipette is very delicate and easily broken. A permanent pipette (Fig 1) can be made by drawing down a piece of 5 mm glass tubing and sealing into the attenuated end

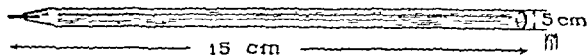


Fig 1—Pipette for antigen with shaft of gold needle sealed into tip

a half inch length of a gold needle shaft of number 21 gauge. The end of the needle after being cut off squarely is polished down until the wall becomes thin enough to allow a drop of antigen of 0.008 cc to be delivered, the size of the drop is determined not so much by the diameter of the opening as by the thinness of the wall at the tip. Such a pipette will deliver the exact amount of antigen indefinitely.

#### HOLDER FOR SLIDES

Fig 2 illustrates a convenient and simple slide holder so that the slides after receiving the serum and antigen can be readily picked up from the table without danger of spilling. The holder is made of any suitable thin metal and is folded as indicated in the figure. The slide should project a half inch beyond the margin of the metal.

#### HEATER FOR PARAFFIN

Rings of paraffin, requiring the use of melted paraffin must be made on glass slides in order to perform the test. To give the best results, paraffin should be at a temperature of about 165° C. Since this approaches the temperature at which paraffin catches fire and since paraffin of this temperature will blister the skin rather badly, an electric heater has been designed (Figs 3 and 4), so

\*From the Section on Clinical Pathology, The Mayo Clinic, Rochester, Minn.  
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that the heated paraffin will be kept at a relatively constant temperature with no danger of being set on fire and no danger of spilling. To make the box, sheet iron of number 16 gauge is folded as illustrated, and the ends welded in. It is insulated on the inside with asbestos, and on this is placed a simply wound heating element of nichrome wire. Suitable steel encased heaters can be purchased on the market with a wattage of 150. Near the heating element is placed a simple bi-metallic thermostat such as is used in heating pads. On the top of this is now placed another sheet metal box with welded ends. Asbestos is packed in around this and a sheet of asbestos is placed on top between the two metal boxes. Once the thermostat is properly adjusted no further care is necessary.

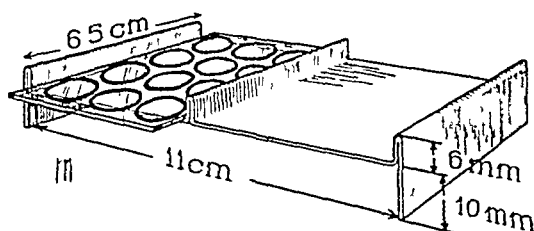


Fig 2—Frame to hold slides

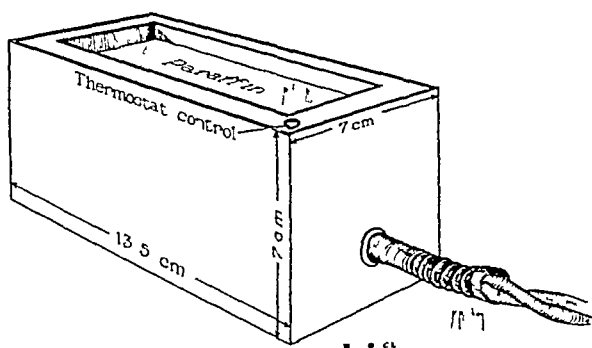


Fig 3—Heater for paraffin

#### MACHINE\* FOR TILTING AND SHAKING SLIDES

After the antigen and serum are mixed, the slides must be shaken, and the edges tilted in rotation without revolution for three minutes, so that all edges of the slide are tilted in rotation every second. The motion imparted to the slide will be clearly described later. When large numbers of these slides have to be used rotation by hand becomes very laborious and tiresome, in addition to that it is impossible to keep up a steady and constant speed of tilting in rotation. Therefore, to perform this act, a machine has been designed which may be understood by reference to Figs 5 and 6 and the following description.

A-A designates two of the four pan-shaped containers or slide holders mounted on the plate B, which is secured in the inner ring of the ball bearing C the outer ring of which is in turn secured in the plate D. Plate D is mounted on and secured to the semicircular piece G mounted in the housing J. The piece

\*This machine was designed and built in The Mayo Clinic machine shop by Mr. George G. Little.

$G$  is graduated at  $G$  for the degree of tilt to which the plate holders  $A-A$  may be set, accomplished by and through the action of the worm thread on the shaft  $H$  meshing in the circular rack of the piece  $G$ . It is turned by the knurled head  $I$ . An angle of  $15^\circ$  is correct for performing Kline tests. The stationary shaft  $F$  is mounted securely in the base of the machine  $Q$  and passes up through the

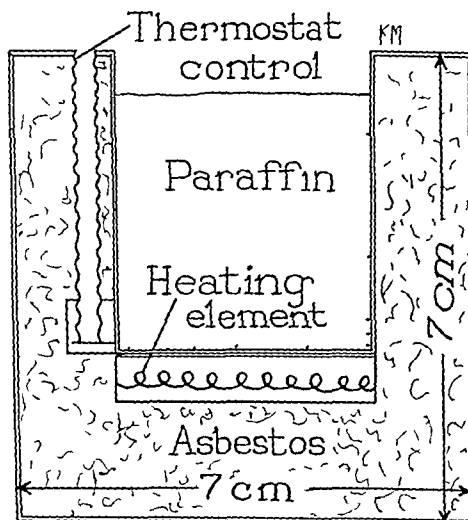


Fig 4—Heater for paraffin cross section

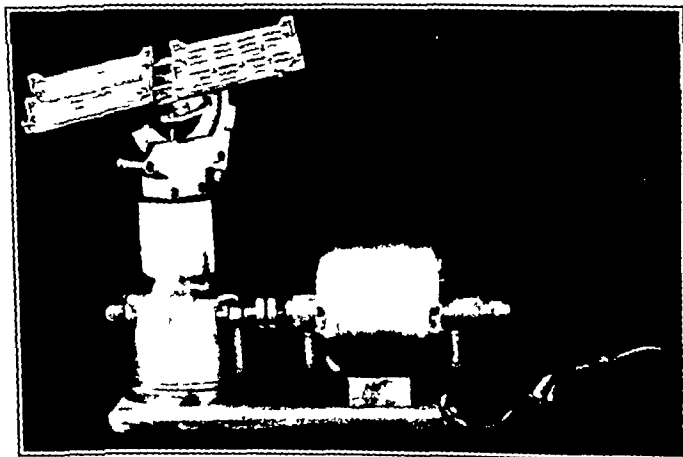


Fig 5—Machine for tilting and shaking slides

hollow spindle or shaft  $K$  to the universal joint  $E$ . The universal joint  $E$  is supported by four pivot screws two being in the yoke at the upper end of the shaft  $F$  and two in the hub or trunnion of the plate  $B$  within the inner ring of the ball bearing  $C$ . The housing  $J$  is secured to the main driving shaft  $K$ , rests thereon, and is caused to revolve when the shaft  $K$  is set in motion. Shaft  $K$  is located in suitable vertical bearings  $T$  upper and  $U$  lower, mounted in the main housing of the machine and supplied with proper long-time lubrication oil

cavities. Shaft *K* is supported endwise with a ball-thrust bearing that sustains the weight and provides antifriction in conjunction with the upper bearing *C*, thus making it possible to use a very small, low-powered motor to drive the machine. Shaft *K* has a worm wheel (not shown) mounted to mesh with and be driven by the worm *N* on the small shaft in the bearings *V* and *W*, the worm *N* is also provided with a ball-thrust bearing *M*. The part *P* is a detail of construction in which is located the lower vertical shaft bearing and facilitates assembly of the machine. The part *R* is a small flexible coupling connecting the worm shaft *N* to the motor *S*, which is a cheap fan motor. In action the glass plates being placed in the holders *A-A*, the worm *H* is turned to bring the holders into the proper or desired angle as indicated at *G*, the motor started to

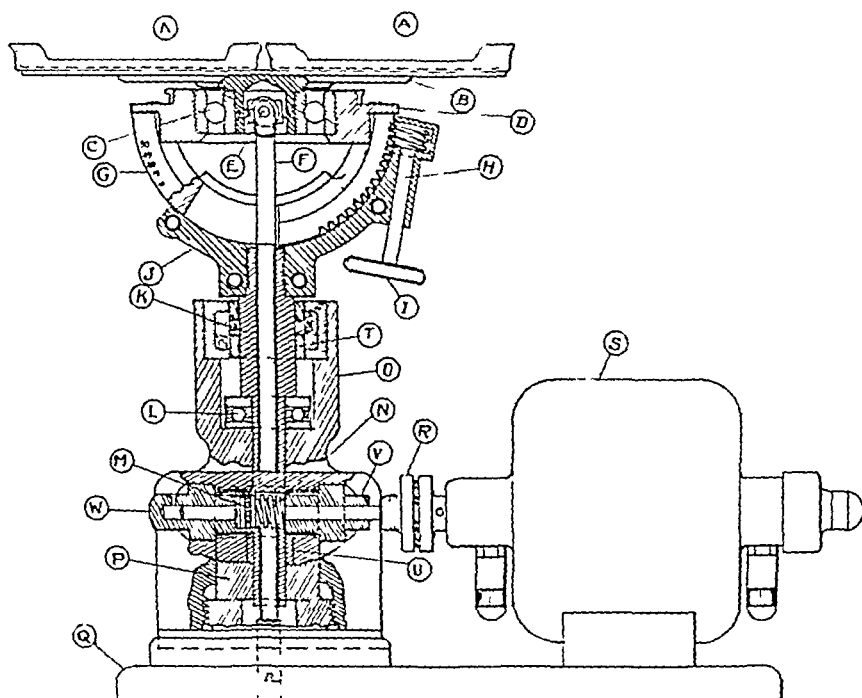


Fig. 6.—Machine in section for tilting and shaking slides

cause the shaft *K* to revolve, carrying the housing *J* with it and also causing the outer ring of the ball bearing *C* to revolve which, due to the fact that the level of zero shaft of *G* and the ring *C*, plate *B* and holders *A-A* have been changed to an angle with the shaft *K* and shaft *F* imparts an oscillating motion to the plate *B*. The inner ring causes the universal joint to assume a like angle, permitting the shaft *F* to remain stationary although the parts *K*, *J*, *G*, *C*, *B* and *A-A* are caused to revolve. It is the action of the universal joint and the stationary position of the shaft *F* holding the plate *B* and the inner ring of *C* stationary that permits the outer ring of *C*, together with *G* and *K* to revolve and impart a continuous tilting motion to *A-A*.

This movement is very simple and is easily understood if one thinks of the end of a round shaft cut off at an angle other than a right angle, then, while

it is revolved in a vertical position, if a rectangular plate is held against the end surface of the shaft, at the same time preventing the plate revolving, the motion imparted to the plate will be like that of the machine herein described

The machine may be used to perform all manner of slide agglutination tests and will give great constancy in performing the Kline tests, both on serums and on spinal fluids

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## THE VALUE OF SITOSTEROL AS A FORTIFYING AGENT FOR BEEF HEART ANTIGEN USED IN THE PRECIPITATION TEST FOR SYPHILIS\*

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B S LEVINE, PH D, CHICAGO, ILL

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IT IS now known that cholesterol is not the only substance which increases the antigenic strength of alcoholic beef heart extract, but that this property is possessed by several of the sterols Eagle<sup>1</sup> has made a study of this subject. He selected 50 water-insoluble alcohol-soluble substances and tested them for their antigen fortifying properties. Nineteen of the substances had to be discarded for technical reasons. Of the remaining 31, 16 proved to possess slight, 7 fair, and 8 very good fortifying qualities. First on the list of the 8 stood cholesterol, a reagent well known to serologists. It was followed by sitosterol. The latter belongs to a group of phytosterols, and as used by Eagle constitutes the water-insoluble alcohol-soluble nonsaponifiable fraction of wheat germ. In conjunction with cholesterol this reagent proved to possess ample sensitization power in Eagle's experiments.

The final reagent used in the experiments referred to consisted of the concentrated beef heart extract to which were added 0.8 per cent cholesterol and 0.8 per cent sitosterol. This supersaturation is accomplished by boiling. Upon cooling, the excess of cholesterol and sitosterol crystallizes out. It is, therefore, necessary to heat the reagent at 56° for some minutes before the antigen-saline suspension can be prepared for use as per titer. Eagle concludes that "the Wassermann reaction carried out with this antigen appears to be the most sensitive of biologic tests," since a 1:10,000 dilution of the antigen sufficed to give a four-plus fixation with a strongly positive serum specimen.

It must be stated at this point that such high degree of fortifying power is not limited to sitosterol, nor is it a result of supersaturation of the beef heart extract with the sterols. On several occasions I have prepared a highly lecitholized Kolmer antigen, fortified by the addition of only 0.2 per cent cholesterol, the primary titer of which was 1:10,000. The Kolmer antigen used in the laboratory of the Institute at this time is 0.5 cc of 1:1,000 dilution, and this, of course, is ten times the primary titer. In view of the above, the statement that "there is reason to believe that this antigen (cholesterol sitosterolized) possesses almost the maximum sensitivity obtainable" and that "any further improvement must await the discovery of better sensitizers," appears unwarranted.

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\*From the Clinical Laboratory, Public Health Institute.  
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The experiments referred to apparently were designed to show that excessive sensitization by a fortifying agent is not a potent danger, and that with the combination of cholesterol and sitosterol, it is possible to continue to increase the efficacy of the antigenic reagent, with no danger of giving falsely positive tests. Such a view must necessarily rest upon the assumption that the complement fixation and antigen precipitation reactions, based upon fortified beef heart extract, are immunologic reactions specific for syphilis. There is still no unanimity of opinion on this point. My practical experience and experimental evidence lead me to the conclusion that the reactions are truly immunologic, but that they are specific for syphilis only when kept within certain zonal limits of reaction.<sup>2</sup> And the degree of fortification of the beef heart extract is one of the factors which determines the safe limits.

Antigen precipitation in syphilis follows in a general way, the type-curve of colloidal precipitation. According to this type-curve, aggregation of the suspended antigen increases to a maximum with the increase in the content of any one of the constituents of the immuno colloidal system of precipitation. Further increase in the content of the particular constituent leads to a progressive reduction in the aggregation of the antigen particles. The continued increase in the fortifying sterol content of the beef heart extract affects the precipitation reaction similarly. Therefore, a point is reached beyond which further addition of the fortifying agent to serums of weaker reaction (keeping the lipid content of the alcoholic extract constant) tends to retain the sensitized particles in a state of dispersion. The force that keeps the particles in dispersion is sufficiently strong to effectively resist the settling power of ordinary centrifugalization. Under such conditions suspended antigenic particles sensitized by immunobodies other than of syphilitic origin being generally of a low concentration, will remain in an invisible state of suspension. The same is true of suspended antigenic particles sensitized by syphilitic immunobodies of low concentration. It is evident, therefore, that in antigen precipitation in which visibility is the sole criterion for the judgment of the positivity of a serum specimen, the danger of obtaining falsely positive results with an excessively fortified antigen is not very great.

The danger of bringing about nonspecific reactions in complement fixation is more potent than in antigen precipitation. The dilutions of the constituents used in this reaction, and of the antigenic reagent in particular, are high. The crest of the curve of sensitization and with it of complement consumption is not reached as readily. Continued increase in the sterol content of the beef heart extract, even beyond the point of normal saturation, continues to widen the zone of reactivity of the antigen. This is accomplished mainly through an increase in the surface area that has become activated to complement consumption. A point is finally reached at which the fixation of complement by antigenic particles sensitized by immunobodies other than of syphilitic origin exceeds one unit, making nonspecific fixation evident.

Abundant experience has shown that sensitization of the beef heart reagent and the proportionate fixation of complement may be of a truly immunologic nature, but may be caused by immunobodies other than those of syphilitic origin. The work of Kolmer indicates this.<sup>3</sup> It is also recognized by the

insistent demand for a "standardized technic" Experimentally this can be demonstrated by a laborious evaluation of the results of a large number of cases<sup>4</sup> A quantitative comparison through a simple technic limited to one serum will prove only the increase in the intensity of the antigenic reaction of the beef heart extract with the increase in the content of the sterols It will throw no light on the specificity of the highly fortified reagent To arrive at the proper conclusion with regard to the limits of specificity in fortified beef heart extract, it is imperative that series of numerous complement-fixation tests be performed in which use is made of the same beef heart base fortified with cholesterol or other sterols from 0.1 per cent up to supersaturation (about 2.0 per cent at 56°)

Experimental evidence proving this point will be presented in a forthcoming report The work reported in this paper is limited to the study of the value of sitosterol and of supersaturation as means for the fortification of antigen used in the precipitation reaction

As a result of his studies on sitosterol as an antigenic fortifier, Eagle developed what he terms a more sensitive flocculation test<sup>5</sup> The test briefly is as follows An alcoholic beef heart antigen is prepared practically in the usual manner and is fortified by a combination of cholesterol and sitosterol to the point of oversaturation One cubic centimeter of the fortified antigen is mixed with 1.3 c.c. of 4.0 per cent NaCl solution This antigen-saline mixture is left at rest for thirty minutes to undergo a process which Eagle calls "ripening" Four hundredths of a cubic centimeter of this antigen-saline mixture is then placed into serologic tubes to which 0.4 c.c. of the inactivated serum is added The tubes are properly marked for identification, and the set is shaken in the Kahn shaker for two minutes Following this, the sets are incubated at 37.5° for four hours or longer The tubes are then centrifugalized for ten minutes at a rate of 3000 r.p.m. One and two-tenths cubic centimeter of saline is then added to each tube, and the results read A comparative study was made of the results obtained by this new precipitation test with several others It was found that the new test possessed some technical advantages over the others but that it did not attain the degree of sensitivity claimed for it<sup>6</sup> Some serums from known syphilitic cases which were positive by the Kolmer and by the highly cholesterolized complement fixation and by the Kahn had to be recorded as negative and doubtful by the new test

Close observation soon convinced me that sitosterol and cholesterol added jointly to the beef heart extract did not increase the sensitivity of the antigen as expected and that supersaturation with cholesterol alone was as efficacious as with a combination of the two fortifiers It appeared, in fact, that saturation of the beef heart extract with either or both of the fortifiers is no requisite for a highly sensitive antigenic reagent, and, further, that supersaturation represents a point beyond the optimum for precipitation

The following experiments were performed to verify the results of the preliminary observations

Sixty-eight cases with histories of syphilis were subjected to the Eagle test using 0.04 c.c. of the antigen-saline suspension and 0.4 c.c. of the inactivated serum The tubes were shaken for two minutes and incubated at 37° for four



hours. They were then centrifugalized, saline added, and the results read and recorded as described in another paper.<sup>7</sup> The same serums were subjected to the following procedure: 0.05 cc of the Kahn antigen-saline suspension was placed into Kahn tubes. To each was added 0.15 cc of the corresponding serum. The set was shaken for two minutes, placed in the incubator for four hours, the tubes were centrifugalized the same as for the Eagle tests and 1 cc of saline was added. The results are shown in Tables I and II.

TABLE I

NO	EAGLE	KAHN 1st	NO	EAGLE	KAHN 1st
1	2	0	35	4	2
2	4	2	36	3	4
3	4	4	37	4	4
4	2	0	38	4	4
5	4	3	39	2	2
6	2	0	40	1	1
7	2	P M	41	1	3
8	4	P M	42	3	2
9	4	3	43	1	1
10	3	1	44	P M	3
11	4	4	45	4	4
12	4	4	46	2	0
13	4	3	47	2	1
14	3	1	48	4	2
15	4	4	49	4	2
16	4	0	50	4	4
17	3	0	51	4	4
18	2	1	52	3	2
19	2	2	53	3	2
20	2	2	54	3	3
21	4	4	55	3	4
22	4	4	56	4	4
23	2	4	57	4	4
24	2	1	58	4	4
25	4	3	59	4	4
26	4	3	60	4	4
27	4	3	61	1	2
28	2	2	62	4	4
29	0	1	63	0	2
30	2	2	64	4	3
31	P M*	2	65	1	0
32	4	4	66	2	0
33	0	2	67	3	2
34	4	4	68	2	1

\*P-M = Plus-Minus

TABLE II  
RECAPITULATION

Total No. of tests	68
Total positives by the Eagle technic	64, or 94 % of total
Total positives by Kahn 1st, centrif	58, or 85 % of total
Plus minus by Eagle technic	2, or 3.0 % of total
Plus minus by Kahn 1st, centrif	2, or 3.0 % of total
Negative by the Eagle technic	2, or 3.0 % of total
Negative by Kahn 1st, centrif	8, or 12 % of total
Total average value, by Eagle technic	2.9
Total average value, by Kahn 1st, centrif	2.4
Average of positives, by Eagle technic	3.1
Average of positives, by Kahn 1st, centrif	2.8

It is seen from Tables I and II that the Eagle technic resulted in the greater number of positives (94 per cent) as compared with the Kahn (85 per cent). The general intensity of the reaction was also greater in the Eagle tests than in the centrifugalized Kahn first tubes (2.9 and 3 as compared with 2.4 and 2.8). However, the ratio of antigen to serum in the Eagle technic is 1:10, while in the Kahn first tube it is 1:3. The effect which the ratio of antigen to serum has upon the efficacy of antigen precipitation has been described by Porges et al.<sup>8,9</sup> They showed that the aggregation of the antigemic reagent follows a type curve as the ratio of the serum to antigen is progressively increased. Kahn showed that his 1:3 ratio, with very few exceptions, belongs to the midpart of the ascending portion of the curve, while the ratio of 1:10, also with some exceptions, is about at the crest of the curve.<sup>10</sup> It is not surprising, therefore, that the Eagle series, with an antigen to serum ratio of 1:10 gave more numerous and more intense precipitation results than the Kahn first tube, which represents a ratio of 1:3.

It is argued in one of the preceding paragraphs that supersaturating the beef heart extract with the fortifying sterols, according to the colloidal type-curve of precipitation, should lessen their original fortifying power in the precipitation test. That is, normally fortified antigen should prove more efficacious than supersaturated antigen in a long series of comparative tests, in which the ratio of the reagent to serum is the same for both antigens. To establish this, the following experiment was carried out. Beef heart powder was extracted to complete freedom from other soluble substances. It was then extracted with

TABLE III

NO	EAGLE	LEVINE	NO	EAGLE	LEVINE
1	4	4	31	P M	4
2	4	4	32	1	4
3	0	1	33	4	4
4	4	4	34	2	4
5	4	4	35	4	4
6	0	3	36	2	4
7	4	4	37	4	4
8	4	4	38	0	2
9	4	4	39	4	4
10	0	2	40	2	3
11	4	4	41	4	4
12	0	4	42	3	3
13	4	4	43	4	4
14	4	4	44	0	1
15	2	2	45	0	4
16	P M	4	46	0	2
17	4	1	47	2	2
18	4	4	48	P M	1
19	4	4	49	P M	3
20	2	2	50	4	4
21	P M	4	51	P M	1
22	0	4	52	4	4
23	4	4	53	0	4
24	4	4	54	4	4
25	4	2	55	1	2
26	4	3	56	4	4
27	3	4	57	4	4
28	4	4	58	2	3
29	4	4	59	4	4
30	1	4	60	4	4

alcohol exactly as for the preparation of the Eagle basic substance. This was fortified with 0.6 per cent cholesterol. The final reagent was not standardized, but was used in the test exactly as prescribed for the Eagle reagent and was used as per Eagle technique. This series is designated in Tables III and IV as the Levine series. Sixty tests were carried out on the same serums by the two techniques. The results are reported in Tables III and IV.

TABLE IV  
RECAPITULATION

Total number of tests	60
Positive by the Eagle technique	45, or 75.0%
Positive by the Levine technique	60, or 100.0%
Plus minus by the Eagle technique	6, or 10%
Plus minus by the Levine technique	None
Negative by the Eagle technique	9, or 15.0%
Negative by Levine technique	None
Average Eagle value on the basis of the total	2.5
Average Levine value on the basis of the total	2.9
Average Eagle value on the basis of positives	3.4
Average Levine value on the basis of positives	3.8

From the comparison of the results of the two sets of tables it is seen that when the ratio of antigen to serum in the two procedures is the same, the antigen containing 0.6 per cent cholesterol yields precipitation results which exceed the results obtained by the Eagle antigen (supersaturated with cholesterol and sitosterol) in number (100 per cent as compared with 75.0 per cent) and in intensity of reaction (2.5 and 3.4 as compared with 2.9 and 3.8). It is evident from this that the use of sitosterol to obtain high sensitivity in precipitation tests is unnecessary. The fact that sitosterol and many other substances possess antigen fortifying qualities becomes of theoretical interest and importance. Since cholesterol is more efficacious as an antigenic fortifier than is sitosterol, as shown by Eagle and by the experiments here reported, and since the object in any laboratory technique is simplification, it is best that cholesterol alone continue to be used as the fortifying agent in the antigen precipitation tests for syphilis.

The results recorded in Tables III and IV indicated that by varying the antigen to serum ratio a suitable diagnostic reaction-level can be attained with the lowest percentage of sterol compatible with unmistakable visibility of centrifugalized positive reactions. Numerous series of experiments were carried out. The following procedure was found the most appropriate for practical laboratory purposes. Beef heart powder was extracted to complete freedom from ether-soluble substances. It was then extracted with alcohol for several days at room temperature. The filtered alcoholic extract was fortified with 0.4 per cent cholesterol and standardized so as to be used in a 1:1 ratio with saline. In the test proper 0.0125 c.c. of the antigen-saline mixture and 0.1 c.c. of the serum are used. No shaking by machine and no incubation at 37° are resorted to. After centrifugalization, 1 c.c. of saline is added to each tube and the results

observed. This procedure forms the basis of a centrifugoprecipitation test now in use in the laboratory of this Institute in conjunction with other accepted tests. The details of the newly developed precipitation procedure will be reported elsewhere.

In Table V a series of results obtained by this procedure are compared with those of the Kahn two-tube precipitation<sup>11</sup> and the Kolmer and cholesterolized complement-fixation.

TABLE V

NO	LEVINE	KAHN	KOLMER	CHOLESTEROLIZED
1	4	4 4	4 4	4 4
2	4	Tr 2	4 4	2 0
3	4	4 4	4 4	4 4
4	4	3 3	4 2	1 0
5	4	4 4	4 4	4 4
6	3	Tr Tr	4 4	4 4
7	3	3 3	4 4	4 4
8	3	Tr Tr	4 4	4 3
9	4	4 4	4 4	4 0
10	4	4 4	2 2	0 0
11	3	0 1	4 4	4 0
12	4	4 4	4 4	4 4
13	4	4 4	4 3	3 0
14	4	4 4	4 4	4 4
15	4	0 0	4 1	0 0
16	2	0 Tr	4 1	0 0
17	4	4 4	4 4	4 4
18	4	4 4	4 4	4 4
19	2	0 0	3 1	0 0
20	3	4 4	4 4	4 4
21	4	4 4	4 4	4 4
22	3	Tr 3	4 4	4 4
23	4	4 4	4 4	4 4
24	4	Tr 3	2 2	0 0
25	1	0 0	0 0	0 0
26	P M	0 0	0 0	0 0
27	4	4 4	4 4	4 4

An equal number of known negative serum specimens were run simultaneously. The positive results in this procedure, no matter what their intensity were distinguished from the negatives with remarkable facility. So easy was the visibility that the results of the tests could be noted and called off faster than the recording person was able to write them down. A consideration of the results of the experiment recorded in Table V together with a study of the histories of the cases from which the serums were taken prove that fortification of the antigenic principle with a cholesterol content as low as 0.4 per cent (as compared with Eagle's 0.8 per cent cholesterol and 0.6 per cent sitosterol) suffices for the detection of syphilis antibody by the centrifugoprecipitation method. It substantiates the conclusions arrived at from the preceding experiments that the use of sitosterol adds nothing of intrinsic value to antigen precipitation, and that saturation and supersaturation of the alcoholic beef heart extract with the fortifying sterols is no requisite for ample sensitivity.

#### CONCLUSIONS

1. Supersaturation of the beef heart extract with sterol fortifiers does not bring out its maximum efficacy in the precipitation test for syphilis.

2 A redispersion of the sensitized particles results from, and a protective influence is exerted by the excess of fortifier which is sufficiently strong to prevent the settling of the sensitized complex through ordinary centrifugalization

3 The addition of sitosterol to cholesterolized antigen is of no intrinsic value in the antigen precipitation test

4 The principle of simplicity in laboratory technique would therefore, seem to demand that cholesterol alone continue to be used as the antigen fortifying agent in the tube-precipitation test for syphilis

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## BLOOD The Neutropenic State, Doan, C. A. J. A. M. A. 99 194, 1932

The author presents a comprehensive yet succinct review of conditions associated with decrease in the number of leucocytes, especially agranulocytosis, presents illustrative cases, and the following conclusions

The neutropenic state may be chronic or acute, constant, recurrent. In diagnosis it must be sharply differentiated from the leucopenia associated with many other clinical syndromes

The chronic condition of moderate leucopenia may either mean a normal physiologic equilibrium maintained at a level somewhat lower than the average, with no detectable influence on the normal health of the individual, or it may reflect a low myelocytic reserve in the marrow with constant potential danger of marrow insufficiency and decompensation

The acute or malignant neutropenic state presents a crisis that is rapidly fatal if recovery of marrow function is not initiated promptly. The underlying pathologic condition may include myeloid hypoplasia or hyperplasia

Experimental and clinical observations form the basis for attributing to nucleic acid and to the nucleotides chemotactic, maturative and initiatory stimuli for neutrophilic myelocytes when the basic mesenchymal tissues from which they arise are in a condition to respond

Blood transfusion and irradiation may act through the same nucleotide mechanism when effective. The latter, because of its potentially destructive affinity for hematopoietic tissues, must be used with great caution when stimulation is desired. Further experimental studies are needed to ascertain whether the x rays may be really primarily stimulatory to myeloid tissue without a preceding destructive phase

## BLOOD Clinical Significance of Volume and Hemoglobin Content of the Red Blood Cell, Haden, R. L. Arch. Int. Med. 49 1032, 1932

The following summary is presented

1 The accurate determination of the volume, hemoglobin content and hemoglobin concentrations of the erythrocyte is a relatively simple procedure and should be carried out in every case of anemia that requires careful study

2 The results are best expressed as indexes rather than in absolute figures. The indexes express the absolute values relative to normal

3 In normal men and women the indexes are always within the limits of normal (0.09 and 1.10)

4 Every case of anemia that warrants thorough study should be classified on the basis of the number, the volume and the hemoglobin content of the red blood cells

5 A simple classification and terminology on the foregoing basis is suggested

6 The typical determinations in different clinical groups of anemia are reported

7 In the case of acute mechanical blood loss there is a change in the number of cells only, in chronic loss the cells become progressively smaller, with a low hemoglobin content

8 If the anemia is due to accelerated destruction the mean cell volume tends to increase since the reticulocytes are usually larger than normal

9 With decreased blood formation the determinations are very variable. The mean cell volume and hemoglobin content are seldom greater than normal except in pernicious anemia

10 In pernicious anemia an increase in volume and hemoglobin content is the most characteristic observation on the blood

11 The volume and hemoglobin content are important aids in prognosis

12 In pernicious anemia the mean volume of the erythrocyte is the best index in treatment. If the patient is adequately treated, the mean cell volume returns to normal.

13 In the treatment for other types of anemia the volume and hemoglobin content of the red cell are valuable aids in treatment.

**RHEUMATIC FEVER** Streptococci in the Blood in, Lichtman, S. S., and Gross, L. Arch Int Med 49 1078, 1932

Study of 5,233 consecutive blood cultures in a general hospital shows that with adequately sensitive methods an incidence of nonhemolytic streptococcemia (alpha and gamma types) between 4 and 15.5 per cent with an average of 6 per cent occurs in at least 9 diseases, i. e. acute rheumatic fever with polyarthritides, chronic rheumatic cardiovascular disease, rheumatoid arthritis, aplastic anemia, pernicious anemia, leucemia, colitis, meningococcus meningitis and pyelitis and pyelonephritis. On the basis of the incidence of the "transient" streptococcemia alone, these organisms cannot justifiably be considered as the causative agents of these diseases.

**BIOPSIES** Suction in Obtaining Endometrial, Klingler, H. H., and Burch, J. O. J. A. M. A. 99 599, 1932

*The method is as follows:*

An ordinary Kevex-Utzman cannula which in reality is nothing more than a rigid hollow uterine sound with perforations at the tip, was used.

While the results with this instrument have proved satisfactory in a number of cases, it was frequently difficult to insert the instrument on account of its rigidity. A Luer or all glass type of syringe was attached to the cannula. However, numerous syringes were broken in withdrawal and suction due to the stress at the junction of syringe and cannula. This made it necessary to substitute a tube constructed of malleable copper and attached to a record syringe.

The technic is briefly as follows:

The patient is placed in the lithotomy position. Cervical exposure is obtained by means of a bivalve speculum. The cervix is cleansed of mucus and the os painted with mercurochrome 220 soluble. The tube is inserted into the uterus and bent slightly, depending on its position. When the cannula comes to rest against the uterine wall, the syringe is attached to the cannula and strong suction is made while slight pressure keeps the tip of the cannula in contact with the uterine mucosa. While suction is maintained, the cannula is withdrawn for about half an inch and then released. The instrument is then rotated slightly so that on reinsertion it will come to rest on another part of the mucosa. This process is usually repeated about three times, and the instrument withdrawn during continuous suction. The apparatus is carefully washed with solution of formaldehyde by drawing the fixative back and forth several times in order to remove all the particles of tissue from the apparatus. It is then carefully washed out and sterilized and is ready for use again.

**CHOLESTEROL**, Blood, in Hepatic and Biliary Diseases, Epstein, E. Z. Arch Int Med 50 203, 1932

Improvement in the accuracy of diagnosis and prognosis of various hepatic and biliary diseases is possible by means of a quantitative study of the blood cholesterol and cholesterol ester.

In obstructive jaundice, hypercholesteremia is usually encountered. It roughly parallels the degree of obstruction and the bilirubinemia and returns to normal with relief of the obstruction. Exceptions are noted in cases of marked cachexia, cholemia and superimposed infections. The cholesterol esters in mechanical obstruction rise concomitantly with the total cholesterol in about half the cases, in the other instances they remain normal but lag relatively behind the increased free cholesterol.

In degenerative diseases of the liver, a pronounced divergence between the bili-

rubinemia and cholesteremia usually occurs, the more severe the damage to the liver, the greater the tendency to hypocholesteremia. This divergence between the hyperbilirubinemia and the cholesteremia offers a means of differentiation from the cases of mechanical obstruction. In parenchymatous degeneration of the liver, a drop in cholesterol esters parallels the severity of the damage even more accurately. In the rapidly fatal cases the cholesterol esters are very low or absent throughout the course of the disease, in less severe cases, the initial low ester values eventually rise with improvement in the condition.

In atrophic cirrhosis of the liver (Laennec), the cholesterol blood pictures remain normal. Variations occur only when hepatitis or degeneration of the liver are superimposed in the terminal stage of the disease.

In cholecystitis and cholelithiasis with no obstruction to the biliary outflow, the blood cholesterol figures are normal or insignificantly elevated.

**PLASMA PROTEIN, in Relation to Blood Hydration, Peters, J, Bruckman, F S, Eisenman, A J, Hald, P J, and Wakeman, M J Clin Invest 11 97, 1932**

#### I Proteins in Acute Nephritis

Serum proteins have been determined 85 times in 38 cases of acute nephritis.

Edema was regularly found when the protein concentration was below 4 per cent, but was sometimes present in the early stages of the disease when protein and albumin were within or just below the normal limits.

In the early stages of the disease edema appears to be due partly to vascular disturbances which increase the hydrostatic pressure or the permeability of the capillaries.

Serum albumin reductions, when they occur, seem to be referable to leakage of albumin into the urine and malnutrition.

Serum globulin in acute nephritis is frequently above normal.

#### II Serum Proteins in Heart Disease

In patients with heart failure serum albumin is frequently reduced.

Although edema of heart failure may occur even when serum protein and serum albumin are at or above the normal level, it is more commonly associated with some degree of albumin deficiency.

The albumin deficits appear to be directly referable to malnutrition.

#### III Serum Proteins in Terminal Stages of Renal Disease

Reduction of serum proteins at the expense of the albumin fraction is common in the terminal stages of renal disease.

The serum albumin deficiency cannot be correlated with the incidence of edema, which is usually referable to heart failure.

Wasting is a characteristic feature of the condition and can be correlated with the serum protein deficiency, unless some other functional disturbance has produced hemoconcentration.

**SERUM DISEASE, Recent Observations in, Hunt, L W J A M A 99 909, 1932**

Serum disease occurred in 28.1 per cent of 2,859 patients who received diphtheria antitoxin, in 22.7 per cent of 878 patients who received scarlet fever antitoxin, and in 81.8 per cent of 55 patients who received antimeningococcus serum.

The occurrence of a serum reaction after the injection of diphtheria and scarlet fever antitoxin is determined in part by the susceptibility of the individual, by the toxic properties of the serum and, in the largest measure, by the total quantity of serum given. Concentrated diphtheria antitoxin calls forth reactions in about the same proportion as does whole serum in corresponding bulk.

The serum reactions after the use of scarlet fever antitoxin were slightly less in frequency than those after the use of diphtheria antitoxin. They were not more severe.

The incidence of serum disease does not vary widely in the various age groups.

The interval between the injection and the appearance of the reaction varies from a few minutes to thirty days. The majority of the reactions appear before the eleventh day.



**TRANSFUSION, Blood, Results of 1500 Transfusions in 1000 Cases, Polayes, S H, and Morrison, M** *Am J M Sc* 184 326 1932

Of 1000 cases receiving a total of 1500 blood transfusions the results were either of doubtful benefit or of no effect whatever in 71 7 per cent

Blood transfusion proved to be a life saving procedure in 14 per cent of the cases Most of these were patients who suffered a sudden loss of a large quantity of blood

Some definite adjuvant beneficial effects were derived from transfusion in 23 3 per cent of the cases

The reaction to the transfusion was unfavorable and the results harmful in 3 7 per cent of the cases

The transfusion was apparently contributory to death in 0 9 per cent of the cases

The excellent results from blood transfusion in the wide variety of diseases as reported by other writers could not be obtained in this investigation Definitely beneficial results should be expected only in the type of case indicated in this study

**ARTHRITIS, Significance of Sedimentation Rate of Blood Corpuscles in Synovial Fluid and Plasma, Kling, D H.** *Arch Int Med* 50 419, 1932

The following technic was used

*Preparation of the Suspension of Blood Corpuscles in Synovial Fluid*—Into a graduated centrifuge tube is put 2 cc of 3 8 per cent sodium citrate solution and then the amount is increased to 10 cc with synovial fluid Into a second graduated centrifuge tube 3 cc of the sodium citrate solution is put, and venous blood is added to 15 cc The amount can be reduced in the ratio of one part of citrate to parts of synovial fluid or blood, the minimum required is 5 cc of citrated synovial fluid and 10 cc of blood, however Six cubic centimeters of the citrated blood is then transferred to a separate test tube and centrifuged at a high speed, simultaneously with the citrated synovial fluid After ten minutes of centrifugation, the tube containing blood is marked at the level of separation of the corpuscles from the serum A second mark identifies the upper level of the plasma In order to be assured that the separation of the cells and plasma is complete, recentrifuge until there is no change in the level of the cells The synovial fluid is centrifuged until the supernatant layer is clear The plasma is then separated carefully from the corpuscles with a pipette, and an equal amount of centrifuged synovial fluid is substituted to the upper mark This procedure secures a suspension of corpuscles in synovial fluid equal to that in the citrated blood

*The Set Up of the Sedimentation Test*

Two 5 cc pipettes are marked at a height of 20 cm from the tip Holes to a depth of about 5 mm are bored in large rubber stoppers (No 10)

The suspension of corpuscles in the synovial fluid and the remainder of the original citrated blood are now shaken until thoroughly mixed Each is then drawn into a pipette to the mark and set up vertically in a rubber stopper, plasticine can be used for this purpose, if desired At intervals of one hour, marks are made at the level of separation of the corpuscles from the clear fluid Final readings are made in twenty four hours.

This method follows the Westergren technic except for two modifications (1) citrate is accurately measured into tubes instead of being drawn into syringes (2) the pipettes are of larger caliber because of the higher viscosity of the synovial fluids Finally, more readings were made than in the routine tests, in order to secure more data

On the basis of a study of 61 cases of arthritis by this method, the following conclusions were drawn

The severity of an infection of the joint is indicated by the sedimentation curve in the synovial fluid, the general reaction is reflected in the blood curve

In acute infectious polyarthritis, the comparative sedimentation index is useful in the determination of the part played by the aspirated joint within the general process

In monarticular arthritis, a high increase in the blood sedimentation and a low sedimentation rate in the synovial fluid indicate foci of infection outside of the joint as responsible for the increase in the sedimentation in the blood

A simultaneous determination of the sedimentation and the viscosity is helpful in differentiating the type of involvement of the joint

Noninflammatory fluids with a low protein content have a low comparative sedimentation index and a low viscosity. This is the case in transudates. On the other hand fluids with a high content of mucin show a low comparative sedimentation index but a high viscosity.

The differentiation of infectious from degenerative types of chronic arthritis by the sedimentation of the blood alone is not possible. The nature of the underlying process can, however, be more accurately discovered by the comparative sedimentation and the viscosity of the effusion.

**Note** This method of comparative sedimentation was also used with other body fluids (pleural, pericardial and ascitic effusions). Four cubic centimeters of citrated blood is sufficient, as these fluids have a low viscosity. Two cubic centimeters is centrifugated and the plasma is replaced by an equal volume of the citrated fluid to be examined. The tests are set up in the standard pipettes for the Westergren method. The findings will be reported elsewhere.

**SCARLET FEVER**, A Survey, Peacock, S. C., Werner, M., and Colwell, C. *Am J Dis Child* 44: 494, 1932.

Of 258 adults examined, hemolytic streptococci were isolated from 72, or 28 per cent and 45 or 17 per cent, gave positive skin reactions.

Forty of the 45 who gave positive skin reactions received toxin for immunization. Of these retested, 87.5 per cent were immunized to the point of negative skin reactions.

On the basis of both time off duty and symptomatology, 15 per cent of the people immunized experienced severe reactions. Approximately 85 per cent of the subjects receiving injections of toxins had more or less distinct reactions.

Hemolytic streptococci had disappeared from the nose and throat of 92 per cent of the subjects surveyed at the end of twenty-eight days. Only 15 per cent of the susceptible subjects, as determined by the Dick test, harbored organisms.

Among the 258 subjects with positive or negative cultures, the number with tonsils and that in whom tonsillectomy had been performed were approximately equal.

The erythema of the skin test averaged 19 by 18.6 mm in diameter, its size bore no particular relation to the grade of reaction experienced by the adults immunized.

Of the adults who gave negative skin reactions, 70 per cent gave no history of scarlet fever, 24 per cent gave a history of having had the disease, and 6 per cent furnished records with no data available on that question.

If the chocolate agar medium is a reliable means of differentiating nonscarlatinal from true scarlet fever streptococci, then 56.9 per cent of the organisms isolated in our series should be classed as related to scarlet fever.

If the opsonification test is of differential value 43 per cent of the organisms isolated were of scarlatinal origin, an agreement of 80 per cent with those giving no reaction on chocolate agar.

Among the 186 persons giving negative reactions to the Dick test, no definite case of scarlet fever developed.

Of the 40 adults giving positive skin reactions, 2 contracted scarlet fever before the series of immunization was completed, and none after the immunizations were complete. Of the 5 subjects who were not immunized, 2 had severe attacks of the disease.

**RHEUMATIC FEVER**, The Contagious Factor in the Etiology of, Collis, W. R. F. *Am J Dis Child* 44: 185, 1932.

The etiology of rheumatic fever can be explained only when its connection with acute sore throat caused by the hemolytic streptococcus is understood. It cannot be described as a contagious disease in the ordinary sense of the word, as more than one factor is responsible for its development. The streptococcal infection of the throat is no doubt contagious in the epidemic sense, but is followed by rheumatic fever only in certain patients. Although there is some evidence in support of the view that before rheumatic fever can develop the patient must have been previously in a prerheumatic condition, characterized by a changed tissue reaction to the organism or its products, this hypothesis must for the present be considered *sub judice*.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughan, Professional Building, Richmond, Va

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### Primary Carcinoma of Lung

THIS is another of those authoritative and exceptionally useful publications which appear periodically as "Medicine Monographs"

The book consists of two portions, the first devoted to general consideration of primary carcinoma of the lung, and the second being devoted to a discussion of the subject from the clinical standpoint

The first six chapters cover the incidence, etiology, histogenesis, classification, metastases, and duration of the disease

From a thorough review of the literature and a rather extensive clinical experience Fried concludes that the more frequent occurrence of cancer of the lung in recent years is more apparent than real and associated with improved clinical and pathologic methods of diagnosis, the increased attention focused upon the condition and the fact that, with the increase in the normal span of life, more people reach the "cancer age"

Bronchiogenic cancer is apparently merely a part of the problem of malignant disease and its true etiology as yet remains unknown

Fried believes that primary carcinoma of the lung is bronchiogenic in origin and follows excessive regeneration of the bronchial tree after inflammatory processes, that it is primarily of basal cell origin, and results from protoplasm of the undifferentiated basal cell of the bronchial mucous membrane

Classification of the tumors is difficult but may be regional, gross, or microscopic the latter being unsatisfactory and largely dependent upon the duration of the disease, which, apparently, runs a protracted course

In the clinical portion of the book diagnosis, laboratory methods, and 47 illustrative cases are thoroughly discussed. The section should prove especially useful because the disease being commonly regarded as rare, is not thought of and so undoubtedly often overlooked

There are numerous excellent and well reproduced illustrations, all photographic

The format is pleasing and the type easily read

This book deserves an enthusiastic reception from the clinician as well as the pathologist and should lead to a better understanding of what is now known to be a far from uncommon disease

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### Human Cancer†

THE subtitle of this book (Etiological Factors, Precancerous Lesions, Growth, Spread, Symptoms, Diagnosis, Prognosis, and Principles of Treatment), indicates its comprehensive scope

As malignant tumors vary in many respects in accordance with the tissue from which they arise as well as the region of the body in which it is located, the arrangement of the book is by regions

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\*Primary Carcinoma of the Lung. Bronchiogenic Cancer. A Clinical and Pathological Study. By B. M. Fried, M.D. Cloth 247 pages 95 illustrations. Williams and Wilkins Co. Baltimore, Md.

†Human Cancer. By Arthur Purdy Stout, Associate Professor of Surgery, College of Physicians and Surgeons, Columbia University. Cloth 1007 pages 331 illustrations. Lea and Febiger, Philadelphia, Pa.

This, for purposes of reference, is both excellent and practical and will undoubtedly be received with favor by those to whom the book is addressed

Each section is preceded by a discussion of the etiologic factors and precancerous lesions as at present understood. This is followed by a clear, succinct, and yet comprehensive discussion of the malignant lesions common to the tissue and region under discussion.

The book is profusely and excellently illustrated.

The recent and pertinent literature is well reviewed and commented upon in the light of an extensive experience. This is particularly notable in the discussions of diagnosis, and of the rapid biopsy methods and the criteria of the degree of malignancy as estimated by tumor cell morphology.

The author lays great stress upon the method of Laidlaw in which, by means of silver impregnation cells may be differentiated in accordance with their embryonic derivation.

This method will doubtless be widely used.

While primarily addressed to surgeons and surgical pathologists, the book is of great interest and appeal to all who come in contact with malignant lesions, and who does not?

The volume deserves a wide circulation as one of the most practical and useful texts at present available.

### Quantitative Clinical Chemistry

THIS volume without reserve may be accepted as the last work to date upon the subject of quantitative methods in Clinical Chemistry and, together with its companion volume, Quantitative Clinical Chemistry, Interpretations, for the first time furnishes a comprehensive and authoritative survey of a field in which many important advances have been made within the last decade or so.

In this book are presented methods for the determination of those substances, found in the body and its excreta, which are of importance for clinical medicine and for which suitable quantitative methods are available.

This, indeed, is an ambitious and comprehensive undertaking, but it has been carried out in a manner eminently satisfactory for those by whom this book will be used as a reference.

Appreciating that laboratory facilities, as well as technical aptitude, are subject to considerable variation in accordance with varying circumstances, the authors have presented in each instance methods of different types as well as, when advisable, both macro and micro methods. The book thus forms a practical manual of technique adapted to practically any and all situations.

The experience and reputation of the authors in this field is a sufficient guarantee of the reliability of the methods chosen by them for presentation, most of which they have, moreover, subjected to extensive practical trial under clinical conditions.

Preceding the technical description of each method is a discussion of the principles upon which the methods are based which is also in effect, not only a critical review of the methods described, but also a cross section of the literature on the subject. The reader is thus informed as to additional methods available in the literature.

The first two chapters on General Chemical Technique and Special Biochemical Technique contain a wealth of practical experience and well repay the perusal of every worker in this field.

Indeed, this is true of all the thirty-two chapters and appendix which compose the book.

There is little doubt but that this volume will be the standard reference text for years to come.

The format and typography are excellent and the authors and publishers are to be congratulated upon the presentation of a volume for which the laboratory worker and the physician have long been waiting.

The book should be on the work shelf of every laboratory in which clinical quantitative chemical determinations are conducted.

\*Quantitative Clinical Chemistry, Volume II, Methods. By John P. Peters, M.D., Professor of Internal Medicine, Yale University, and Donald D. Van Slyke, Ph.D., Sc.D., Rockefeller Institute for Medical Research. Cloth. 1935. 4 figures. Williams & Wilkins, Baltimore, Md.

## The Chemistry of Tuberculosis

CONTRIBUTIONS to the study of tuberculosis in all its phases have been so varied, so voluminous and so widely scattered in the literature that it is only by means of such comprehensive discussions and reviews as are embodied in the present volume that the student of this subject can hope to keep abreast of its many advances

The present edition has been so completely and thoroughly revised as to constitute, to all intents and purposes, a new book

The book is divided into three main sections. The first, devoted to the Chemistry of Acid-Fast Bacteria presents in a comprehensive and authoritative manner all that is known at present of the metabolism of the tubercle bacillus and its relation to the phenomena of the disease

In the second section, The Chemical Changes in the Tuberculous Host, is summarized, reviewed, and discussed, in both an orderly as well as an understandable manner the fruits of modern investigation of the manifestations of the disease correlated with the material discussed in the preceding portion of the book

The final section is concerned with the Chemotherapy of Tuberculosis, discussed and evaluated on the basis of "biologic experimentation carefully coordinated with constructive chemical manipulation and chemical research of an advanced type"

The volume may be regarded as not only a distinct and valuable contribution to a most important subject but as indispensable for a thorough understanding of a complicated subject

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## Clinical Endocrinology of the Female†

ONE of the most recent developments of modern medicine has been the gradual evolution of a beginning understanding of the many complexities involved in the interlocking functions of the group of structures comprising the so-called "organs of internal secretion"

So extensive have been the studies in this field within recent years and so great has been the value and importance of their practical application that in order to understand and apply them a new department of medicine, endocrinology, has come into being

As is usual in the newer developments of medicine, much of the material from which present conceptions of endocrinology arise and upon which its practical applications are based, has appeared in more or less accessible and more or less specialized journals. The present volume by Drs Mazer and Goldstein is eagerly welcomed as presenting under one cover a comprehensive and authoritative survey of endocrinology in its relation to the female, not only from the theoretical but also from the viewpoint of its practical application to the study

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\*The Chemistry of Tuberculosis. By H. Gideon Wells, M.D., Professor of Pathology, University of Chicago, and Esmond R. Long, M.D., Professor of Pathology, University of Pennsylvania. Cloth, ed. 2, 481 pages. Baltimore, Md., Williams & Wilkins Co.

†Clinical Endocrinology of the Female. By Charles Mazer, M.D., F.A.C.S., Assistant Professor of Gynecology and Obstetrics, Graduate School of Medicine, Univ. of Penna., and Leopold Goldstein, M.D., Demonstrator of Obstetrics, Jefferson Medical College. 518 pages with 117 illustrations. Cloth. Philadelphia, and London, W. B. Saunders Co., 1932.

and management of such conditions as are directly or indirectly related to disturbances of the endocrine system

Few conditions more commonly confront the practitioner at large than those related to disorders of the menstrual function, and the phenomena incidental to the menopause, while the number of those suffering from minor gynecologic disturbances is legion

That in many such instances endocrinologic treatment has much to offer is generally known. But exactly *what* it offers and how or by what methods results may be achieved has been discoverable only by search of the literature

This situation is changed by the appearance of this volume. Here the practitioner will find available all the proven and acceptable methods presented in a clear cut and understandable manner

Both the arrangement of the book and the manner in which the material is presented may be highly commended. Comprehensive without being verbose, succinct but not laconic, clear and without ambiguity, despite the complexities of the subject the book is easy to read and to understand

The long experience of the authors and their connection with research in this field assures the reader that their presentation is authoritative

Well illustrated and excellently printed, this volume may be recommended without reserve to the practitioner at large as well as to those whose interests are more specialized as an authoritative contribution to a complicated and very important subject

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## EDITORIAL

### What Price Doctors?

FIVE years ago a committee was organized by physicians and other interested persons to study and report upon the problem of the costs of medical care. The committee numbers around sixty, of whom about one-half are physicians, representing the practice of medicine, public health and industrial medicine, and the remainder are members of the dental and nursing professions, economists, sociologists, and representatives of insurance companies, charitable organizations and endowments and of the public at large. The committee has been headed by Ray Lyman Wilbur, himself a physician.

The devastating effect of illness upon the family pocketbook is obvious justification for such an investigation. This Committee, in close collaboration with The American Medical Association and several other organizations, is in the midst of a very detailed investigation of the entire subject. Booklets reporting their observations in the various fields are being published as the individual studies are completed, and a most valuable accumulation of facts is being made.

Very soon after the organization of the Committee, a familiar psychological phenomenon made its appearance. The doctors had on their own initiative started this survey of their own organization to determine if possible what faults there might be and what might be the most reliable remedies. At once, non-medical reformers took up the cry. Each felt that he alone saw all the shortcomings of the organization of the medical services, and he alone could present the proper solution. The popular magazines were delighted to publish the articles which were usually based primarily on personal experience with illness in the writer's family or personal grievances, and which rarely showed evidence of any wide conception of the intricacies of the problem.

The doctors in general were content to ignore many of the invectives of these articles and to await the more intelligent and better informed reports of the Committee, which would be based on an accumulation and interpretation of facts rather than upon theory and fancy.

The objectives of the Committee have been (1) the accumulation of data on the needs for medical services, (2) data on the existing facilities, (3) study of the cost of adequate medical service, (4) investigation of the reason for the recently increasing costs of adequate care, (5) promulgation of a constructive program directed toward lowering the cost of medical care and (6) stimulation of interest in the problem on the part of the medical profession. Throughout the five years, reports have been made at frequent intervals covering various phases of the economics of sickness and medical practice, with no attempt being made to prematurely suggest radical changes or solutions. The work so far has essentially represented the taking of an inventory.

Most excellent work has been accomplished by the committee and a wealth of statistical data has been collected which can now be substituted as facts, for the previous guesswork of the theorists above mentioned. Whether this will bring us any nearer to a solution of the age-old problem of the economic distress associated with disabling illness is a question that only the future can answer. We look for no panacea and possibly no radical changes in present methods resultant on the investigation of the Committee, but they have already given us an abundance of facts upon which to cogitate and have pointed out some very definite situations obviously requiring correction.

The customary reply of the physician to the criticism that the costs of illness are too high, is that it is the other requisite services and materials and not the doctor's fee, that are responsible. Certain it is that in disabling illnesses requiring hospitalization and nursing care, the doctor's fee comprises a relatively small amount of the total cost.

Each of the agencies involved can however with equal justice make the same statement. Drug bills are often high but if the druggist were to depend for his income entirely on the profits of his prescription service he would not prosper. The cost of a special nurse is ruinous to a meager pocketbook, but no nurse manages to do much more than make a decent living and far too many are unable to lay aside enough to keep them from want after their relatively early retirement. Hospital charges seem tremendous but in spite of this most of them require endowments and it is exceptional even for the private institutions, to achieve what any business man would term an adequate return on his investment. Obviously,



therefore, all sources of expense to the patient may well be investigated, and it is possible that need for cutting expense may be found in any or all of these sources, or it may be found that the defect does not lie in any, but in the present day haphazard manner of providing for the possible future contingency of illness.

For the present discussion we shall leave the problem of other sources of expense to others, perhaps better qualified to discuss them, and shall confine ourselves rather to the question, are physicians' fees too high, is the physician receiving too large an income for his services?

R. G. Leland, Director of the Bureau of Medical Economics of The American Medical Association has made a survey of the incomes of physicians in the United States. It is based upon a representative series of 6,328 doctors, including those in general practice and those in the various specialties. Gross incomes ranged from as low as under \$500 to as high as \$160,499. The survey appears to represent a quite reliable cross section of the incomes of physicians in the United States. Of the 6,328, fifteen reported a gross income of under \$500 and 165 of over \$30,500. One reported a gross income of \$160,499. The vast majority, nearly 6,000, reported gross incomes of between \$500 and \$30,500.

In medicine as in most other fields today the net income is considerably lower than the gross. The physician's overhead is not at all inconsiderable. Leland found that the average net income of the general practitioner is 67 per cent of his gross income. In the specialties where expensive equipment and larger offices are requisite, the net income falls proportionately.

The average gross income for all physicians, including the specialists and those who reported unusually large incomes, was \$9,764.40. The physicians in general practice reported an average gross income of \$7,781 with a net of \$5,250.

The specialties of medicine showed distinct variations in gross incomes. The highest average was in surgery (\$16,393) which was followed closely by roentgenology (\$16,083). Following this, in order, came orthopedic surgery, dermatology, obstetrics and gynecology, toxicology, internal medicine, eye, ear, nose and throat, urology, pediatrics, pathology, insurance work, anesthesia, bacteriology, industrial medicine, general practice, neurology and psychiatry, tuberculosis, physiotherapy, teaching and public health.

Of the entire number of physicians approximately 20 per cent reported gross incomes of under \$4,500. Fifty per cent showed gross incomes under \$7,500, and 80 per cent had a gross income of under \$13,500.

Let us compare present day doctors' incomes with those of ancient times. Galen, the leading Roman physician of his time, who lived in the second century after Christ, is reputed to have received a fee which would correspond in our present monetary standards to \$6,000 for one consultation. The favorite physician of the Arab, Harun-al-Raschid, who reigned from 786 to 802 A. D. was paid \$1,500 annually for "bleeding and purging the Commander of the Faithful." This was in addition to his monthly salary of \$2,500 and a New Year's Purse of \$6,500. His income therefore corresponded to an annual income today of \$38,000. He, himself, estimated his entire fortune in fees as \$10,000,000. He was banished for one reason or another but was recalled to heal El-Meismun, for which he received a fee of \$125,000. During the reign of Charles VIII, Thierry

de Heiv stated his income as 30,000 francs. Money at that time had about eight times the purchasing value of money today.

In the eighteenth century the incomes of doctors scattered over Europe appear to have ranged from \$3,000 to \$90,000 annually. Katherine of Russia paid the equivalent of \$50,000 to have herself and her son vaccinated against small-pox, allowed an additional \$10,000 for travelling expenses, and bestowed upon the physician a life annuity of \$2,500.

Average incomes in bygone days may be guessed at from the following. In 1224 Frederick the Second in Sicily issued an edict requiring physicians to treat the poor for nothing. They were allowed to charge thirty-five cents a visit for office practice and for patients living inside the city. For calls made outside the city \$3.00 could be charged if the physician paid his own expenses, while if the expenses were met by the patient, the fee was \$2.25. It is said that the purchasing power of money at that time was from fifteen to twenty times that of the present. During the reign of Henry VII, Henry VIII, and Queen Elizabeth the Court Physicians received money valued at \$200 annually. Money had about eight times its present purchasing value. In Germany at that time the consultation fee of physicians was \$2.50.

The average cost of a good medical education runs in the neighborhood of \$10,000. To this should be added the value of the money which the student would have been making during the years of his instruction were he not at school, and interest incurred in defraying the expenses of his education. With this the total cost becomes more nearly \$20,000.

If we are to compare physicians' incomes with incomes from other businesses and professions we must discuss it in the terminology of capital and labor. First, reduction must be allowed for a fair interest on the original investment for education of from \$10,000 to \$20,000. Fair interest on an investment as business sees it today reaches more nearly 10 per cent than 6 per cent. From \$1,000 to \$2,000 should therefore be subtracted from the physician's net income before his income can be compared with that of the average man in the white collar class. He should also be allowed a 50 per cent increase for "overtime," for those hours in the day and the night during which he works far past the period of the customary eight hour day.

While the average doctor works hard for what he gets, probably harder than any other one group of individuals, his reward is that he makes enough to live in reasonable comfort in a social environment comparable to that of his clientele. This generalization holds all the way along from the country doctor who is a respected member of his community, whose income is small and expenses light up to the highly paid specialist. Few physicians succeed in doing more than this. Most die with lamentably small estates.

But it is the exceptional man with an unusually large income such as the \$160,000 a year man mentioned above who catches popular fancy and who becomes the subject of a controversy in which unfortunately all the members of his profession are called to account.

It would appear that the occasional very high fees obtained today are not at all unusual in the history of medicine and are quite comparable to ancient experience. The average fees of today are not at all out of proportion to those of

the past. Indeed, this is what one would have anticipated, inasmuch as "physicians' services" is a commodity which has been used or purchased for so many centuries that its average value has been thoroughly standardized. Disproportionate prices may often be obtained for novelties, innovations and luxuries but not for staples. A fictitious value could never have stood so long. Indeed, when one receives his plumber's bill or the electrician's statement one is inclined to marvel at the reasonableness of the physician's charge. Unfortunately the physician is dealing with an intangible service and all that the patient has to show for it afterwards, is more or less return toward health, a condition which he naturally feels should be his anyhow. As with foodstuffs the patient has used up his commodity as rapidly as purchased. Unlike the case with food, he has been unable to enjoy it for the short time he has had it.

The Committee has been able to supply some very interesting basic observations.

In rural Saskatchewan the financial prospect for physicians is so precarious that several of the rural municipalities or counties have found it necessary to subsidize physicians. The annual salaries of these full time physicians ranged from \$3,000 to \$5,000. The median salary was \$4,000. Many of the physicians earned other incomes from special services. For example one municipal physician employed at an annual salary of \$4,000 earned a net income of \$4,858.

Here is a section of North America in which a physician is a necessity and in which we may assume that the community is paying as little as it can to hold the doctor. It has found from experience that it must pay from \$4,000 to \$5,000 or have no doctor. We may therefore take a net income of somewhat over \$4,000 as being the actual basic worth of a physician in a civilized frontier community. The better the economic status of the patient, whether he be rural or urban the more he will insist upon better medical ability and additional services, all of which mean more expense to the doctor. The basic value of the physician to his clientele will therefore increase proportionately with the improved economic status of the clientele.

Or, assuming that the physician's basic value remains the same (\$4,000 to \$5,000 net) his potentialities for increased income will depend upon his opportunities for increased work. Assuming 300 working days to the year, the municipal physician of Saskatchewan made his basic income from an average of three to five office calls and one to two house visits daily. A physician doing more than this basic amount of work should be able to add the value of the additional work to his basic income.

The Endicott Johnson Corporation, one of the world's largest manufacturers of shoes and tanners of leather, provides a complete medical service for its workers and their families. This service, including hospital facilities, is excellently organized, and in 1927 employed 26 physicians. There were 51 physicians outside this service in the town in which the plant is located.

The net income of physicians employed by the factory range from \$3,000 to \$12,000. The net income of those in private practice in the same community ranged from under \$3,000 to more than \$35,000. The analysis of the two sets of incomes is most interesting, showing as it does a distinct parallelism between the two groups, with an important exception in the group not connected with the

factory there are a few physicians who make very small incomes and a few with relatively large incomes. Neither of these extreme groups appear among the medical employees of the corporation. One might interpret this as indicating that among the outside doctors, those with greatest ability may work harder because of the incentive of larger financial reward, and that those without ability are not being paid more than they are worth. Also, that in the factory group the establishment of a top salary of \$12,000 may tend to kill initiative and enthusiasm and a lowest salary of \$3,000 may tend to support the laggard.

In the factory group 46.2 per cent of the physicians received net salaries under \$5,000. In the outside group 35.3 per cent had net incomes under \$5,000. In the factory group 38.4 per cent had incomes between \$5,000 and \$11,000, in the outside group 41.2 per cent. For incomes between \$5,000 and \$13,000 the factory group holds 53.8 per cent, the outside group 54.9 per cent. This was a remarkable parallelism. In other words the difference is seen chiefly at the extremes. Nine and eight-tenths per cent of the outside physicians had net incomes under \$3,000 while 6 per cent had net incomes over \$15,000.

Two apparent facts emerge from these figures, first that independent practice offers the possibility of higher reward for one of unusual ability and second, that the doctors of average ability received comparable incomes in the two groups. There is little likelihood that a great corporation is going to pay a large number of physicians more than it feels they are worth. So, we have another basic value for physicians, this time in industrial communities. The value here ranges from \$3,000 to \$12,000 net, with 54 per cent of them receiving over \$5,000. Most of these are in the \$5,000 to \$7,000 class. If this latter class is combined with the \$3,000 to \$5,000 class, we find that 80.8 per cent receive a net income of between \$3,000 and \$7,000, let us say, average \$5,000. This net income of physicians employed by the Corporation is enlarged by certain perquisites such as eligibility to sick benefits, assistance in the purchase of a home, participation in an investment savings organization and old age pensions.

While the figures quoted are those of the Committee on the Costs of Medical Care the interpretations are our own and we realize that others might derive entirely different conclusions from the same figures, but the parallelism between three sets of figures is both striking and instructive. What is the basic value of the ordinary, so called garden variety of practicing physician, measured in terms of net annual income? Farmers and others living in rural Saskatchewan value his services at between \$4,000 and \$5,000. A large industrial corporation in New York State places his value around \$5,000, actually slightly under this figure. The general practitioners' net income, computed by The American Medical Association is \$5,250.

This being the reward for average ability among physicians, a man with exceptional ability has the right to claim a proportionately larger reward if he so desires. The increase is usually obtained from those well able to afford it. Undoubtedly there are individual instances in which the charge is unjustly high but the cause lies as often at the patient's door as at that of the doctor. Who has not heard the patient's anxious relatives urge upon the physician that "he wants the best of everything regardless of the cost" only to be bitterly criticized later by the same individual because of the expense. When an unjust charge has been

made, nine times out of ten it will be rectified by the doctor if his attention is called to it

From the above review of facts it would appear that by and large the doctor is not overpaid. In some of the specialties those branches of medicine in which the method of therapy is impressive or spectacular, the reward is disproportionate to that in other specialties when weighed in terms of ability or services rendered. But even here the figures accumulated by Leland reveal that the average incomes are not exorbitantly high and are in fact pitifully low when compared with those of business executives and others with comparable responsibilities.

We have made out a case for the doctor because he is our present interest. Undoubtedly others could do equally well for the nurse, the hospital and the druggist. If this be true it would appear that the solution to the problem of the high cost of sickness lies elsewhere. Possibly the most important factor is that in this easy-going country of optimists each person believes that he and his family, contrary to the general experience, will manage to escape the scourge of illness. As in La Fontaine's fable of the grasshopper and the ant, we hop around all summer making no provision for the hardships of the winter.

The problem of the costs of medical care will remain with us until Americans provide for the future instead of living upon the future, or until some form of sickness insurance receives general adoption. By sickness insurance we do not intend to imply the type provided by fraternal organizations or governmental insurance. The experience of the last few years has amply demonstrated the drawbacks of methods of insurance in which salaried physicians render professional services. Without the personal stimulus of competition the exercise of ability tends to become sluggish, among doctors equally as much as among all other types of individuals.

—W. T. T.

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## CLINICAL AND EXPERIMENTAL

### A STUDY OF THE LACTOBACILLI WITH SPECIAL REFERENCE TO THE BACILLUS ACIDOPHILUS\*

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THIS investigation was originally undertaken to determine the potency of the commercial preparations of *Bacillus acidophilus* which are offered for sale on the American market. It was felt that the claims of the manufacturers, as described in the printed literature accompanying their viable preparations, were somewhat exaggerated and a scientific investigation of these statements would be of value to both the medical profession and the public in general. Preliminary examination of the various strains of bacteria isolated from numerous commercial preparations indicated that through proper cultivation any member of the lactobacilli group will assume the morphologic and cultural characteristics of any other desired bacillus of this group. This investigation was then extended to include a study of the lactobacilli group as a whole. It was found that any of the lactic organisms obtained from the gastrointestinal tract could, by employing the proper food supply, be changed into an organism having the morphologic and cultural characteristics of any desired member of the lactobacilli group. The change took place through a definite cycle and depended upon the nature of the food on which the organism was cultured, the  $P_H$  value of the media and the incubation temperature.

As early as 1908 Rodella<sup>1</sup> claimed that the Boas Oppler bacillus was identical with *B. acidophilus* and *B. bifidus* of Tissier. Heinemann and Hefferan<sup>2</sup> employing organisms obtained from sour and aromatic foodstuffs, cattle foods, milk and fermented milk purchased on the open market manured and unmanured soil, normal saliva, normal gastric juice, gastric juice in the absence of

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hydrochloric acid, and human, bovine, and horse feces, concluded that *B. Boas*, *B. Opplei*, *B. panis fermentati*, *Streptobacillus lebensis*, *Leptothrix buccalis*, *B. acidophilus*, and *B. bifidus* were all identical with *B. bulgaricus*. The only objection that could be raised to the above investigation was the failure of the authors to employ a sufficiently large variety of cultural criteria. Moro and Rahe<sup>3</sup> described three varieties of *B. acidophilus* which they termed A, B, and C. Variety C they reported identical with *B. bulgaricus*. Variety B was a streptobacillus exhibiting the characteristics of *B. acidophilus*. Moro. Variety C was a long bacillus or streptobacillus which was similar to *B. acidophilus*, Finkelstein. Kulp and Rettger<sup>4</sup> concluded that the difference between *B. bulgaricus* and *B. acidophilus* constituted sufficient evidence for their classification as separate species. Kopeloff,<sup>5</sup> however, suggested that the gram-positive rods be called *Lactobacillus bulgaricus* and the *Lactobacillus acidophilus* be placed in the same species, with *L. acidophilus* as the central type. *L. bulgaricus* may then be considered a variant of this type, the variation being due to long culturing in milk, and its chief differential characteristic being its loss of ability to develop in the intestines, even when supplied as a milk culture.

A. I. Kendall<sup>6</sup> showed that the chemical action of bacteria depends in a great measure upon the medium in which it exists. Giuseppe Solaimo<sup>7</sup> demonstrated the presence of a gram-negative phase in the development of gram-positive bacteria. Hadley<sup>8</sup> and Hauduroy<sup>9</sup> reported that bacteria have a stage in their development in which they become too small to be recognized by microscopic examination. In this form they were observed to pass through porous filters and were for a time non-cultivable by ordinary methods. They may be made to develop into normal forms and in some cases weeks and months were required to affect this reversion. These various phenomena were observed in the present investigation and form the basis for the development of the cycle through which the lactobacilli pass.

Metschnikoff<sup>10</sup> claimed to have implanted *B. bulgaricus* in the human intestine but later investigations of Kopeloff,<sup>5</sup> Kulp and Rettger,<sup>4</sup> Kulp,<sup>11</sup> Rettger and Cheplin,<sup>12</sup> Clendenning,<sup>13</sup> Cruickshank,<sup>14</sup> and others<sup>26, 27, 28</sup> showed that this was not the case but *B. acidophilus* could be implanted. Cruickshank and Berry<sup>15</sup> were undecided as to whether actual implantation took place or whether the organism merely survived the exposure to acid. Hitziot and Lynch<sup>16</sup> found that in combination with lactose, beneficial results were obtained in *B. acidophilus* therapy but "Implantation was obviously not achieved." James<sup>17</sup> (1927) stated that implantation was impracticable from the use of any commercial preparations examined by him at that time. H. V. Hughes<sup>18</sup> described the installation of living cultures of *B. acidophilus* directly into the colon and observed only an immediate temporary change of flora. This was the only recorded experimental procedure employing direct inoculation. In the other cases, the organism was subjected to the salivary and gastric fluids, thus introducing conditions which would allow a possible change in the morphology and growth characteristics. R. P. Smith<sup>19</sup> found that a change of flora over to the aciduric type did not influence the growth of the typhoid and paratyphoid organisms but it did suppress *B. coli*. Our results indicated that in the case of the lactobacilli, implantation could not occur with respect to a specific member of this group if the

conditions were favorable for the development of any other member and were foreign to the original bacillus. It is true they may survive as long as the artificial media containing the specific food substances for their characteristic development is provided, but by preference they will revert to the type that is characteristic of the surrounding media. Kendall has shown that many strains of bacteria produce nontoxic end products in carbohydrate media, and when grown aerobically, but under adverse conditions, they can become anaerobic and utilize the amino acids for food with the consequent production of toxic substances. Thus *B. diphtheria* in a carbohydrate medium grew aerobically and produced nontoxic lactic acid, while in a media containing proteinous products it grew anaerobically and produced the highly active toxic substance, diphtheria toxin.

Our results indicated that if *B. acidophilus* were introduced into the small intestine, only a relatively small number of the bacilli would become implanted by reverting over to an organism having the characteristics of another member of the lactobacilli group whose natural habitat is the adult human intestine. This inversion may cause the harmless *B. acidophilus* to become a toxic producing organism.

## EXPERIMENTAL

The strains of the lactobacilli employed in this investigation were obtained from six different sources and purported to be *Lactobacillus acidophilus*. The source and fermentation characteristics of these strains, before purification, are recorded in Table I.

TABLE I

STRAINS	** 1	** 2	** 3	4	5	6
Source	Commercial paste	Commercial chocolate covered cubes	Commercial cubes	Special labora- tory strain used for com- mercial prepa- ration	Special labora- tory strain used for com- mercial prepa- ration	Isolated from infant feces
Sugars						
Raffinose	++	++	++	++	+	++
Sucrose	++++	++++	++++	++++	++	+++
Lactose	++++	++++	++++	++++	++	++++
Maltose	+++	+++	+++	+++	+++	+++
Inulin				++++	+++	
Glucose	+++	++++	++++	+++	+++	++
*Mannitol	none	none	none	none	none	none
*Sorbitol				none	none	
Dextrin				none	none	
Xylose	++	++	++	++	++	none
Saline				none	none	++
Dulcitol	none	none	none		none	

\*Filtered

\*\*Labeled to contain millions of viable bacteria

The six strains of Table I were purified for use in this investigation in the following manner. Strains 4 and 5 were plated out in tomato whey agar. A characteristic colony selected and transferred to tomato whey broth. The bacillus was again plated out on tomato whey agar, washed and transferred to



tubes of tomato whey and whey agar. Strain 6 was obtained by the Cruickshank and Berry technique and plated on tomato whey agar. A characteristic colony was selected and the organism isolated by means of the modified Barber's technique. It was then grown in tomato whey, transferred to tomato whey agar, fished and inoculated into tomato whey broth, and again fished and transferred to whey agar. Strains 1, 2 and 3 were obtained by filtration and emulsification in sterile saline, plating on tomato whey agar, fishing the large and small (pin head

TABLE II  
CHARACTERISTICS OF THE PURIFIED STRAINS

STRAIN	1	2	3	4	5	6
<i>Morphology</i>						
Size	2 $\mu$ dia	2 $\mu$ dia	2 $\mu$ dia	1 $\times$ 5 $\mu$	1 $\times$ 5 $\mu$	1 $\times$ 6 $\mu$
Form	circular	circular	circular	rods	rods	rods
Arrangement	singly and chains	singly and chains	singly and chains	end to end	end to end	end to end
Motility	none	none	none	none	none	none
Gram staining	pos	pos	pos	pos	pos	pos
Remarks	three elements in chain					
<i>Cultural Characteristics</i>						
Growth	surface	surface	surface	surface and deep	surface and deep	surface and deep
Milk	coagulation	coagulation	coagulation	coagulation and separation of whey in 172 hr		
Litmus milk	acid growth	acid growth	acid growth	acid in 72 hr		
Whey				growth and sediment appearing at the end of 72 hr		
Russel's media				growth in butt		
Tomato agar	small, grayish white colonies			small, smooth, raised colonies 0.7 to 1.0 $\mu$ dia		
Lactose broth	growth, singly, pairs, and in chains of three					
Acidity	acid reaction			strong acid production		
<i>Fermentation</i>						
Raffinose				++	+	++
Sucrose	++++	++++	++++	+++	+++	+++
Lactose	++++	++++	++++	++++	++++	++++
Maltose	++++	++++	++++	+++	++++	+++
Glucose	++++	++++	++++	+++	+++	+++
Galactose	+++	+++	+++	++++	++++	++++
Dextrin				none	none	none
Mannitol				none	none	none
<i>Gas Production</i>	none	none	none	none	none	none

and pin point), grayish-white, translucent colonies to tomato whey, diluting 1:100 with sterile distilled water, and replating in whey agar. Characteristic colonies, of both the pin head and pin point types, were then selected and the organism isolated by the Barber-Chamber technique.<sup>24</sup> The inoculation was made in whey, transferred to tomato whey, and finally grown in whey agar.

The morphology and cultural characteristics were then determined for the various strains and are recorded in Table II. The organisms from the two

TABLE III

DEVELOPMENT OF THE PURIFIED STRAINS OF LACTOBACILLI ON SELECTIVE MEDIA

STRAIN	4	5	6	4	5	6
MEDIA	TOMATO DIGEST (DEXTROSE)			TOMATO DIGEST (LACTOSE)		
MEDIA ROTATION	1	1	1	2	2	2
<i>Morphology</i>						
Size	$1 \times 8\mu$	$1 \times 7\mu$	$1 \times 8.5\mu$	$0.8 \times 5\mu$	$0.8 \times 5\mu$	$0.8 \times 4\mu$
Form	rods	rods	rods	rods	rods	rods
Arrangement	pairs and chains	pairs and chains	pairs and chains	singly and chains	singly and chains	singly and chains
Motility	none	none	none	none	none	none
Gram staining	pos	pos	pos	pos	pos	pos
Remarks	A few take the gram stain poorly			Often chains of 8 elements are found		
<i>Cultural Characteristics</i>						
Growth	best anaerobic			best anaerobic		
Milk	acid with coagulation and no digestion of clot			clotted in 72 hr with whey slowly separating		
Litmus milk	acid with coagulation			acid with coagulation		
Butter milk	slight growth			no growth at end of 72 hr		
Tomato milk	gram negative forms			no growth		
Whey	no growth			no growth		
Kulp's to mato	no growth			no growth		
Tomato digest	gram pos and neg rods			growth		
S and W me dia	growth			growth		
S and W digest	growth			growth		
S and W sucrose	very slight growth			slight growth		
Whey agar	no growth			fair growth		
Nutrient agar	no growth			slight growth		
Broth	very slight growth			very slight growth		
Lactose broth	poor growth			poor growth		
Glucose broth	abundant growth			abundant growth when acidified with acetic acid		
Acidity	about 1 per cent as acetic			about 10 per cent in 72 hr as acetic (very high)		
<i>Fermentation</i>						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	+			++		
Sucrose	+			++		
Dextrin				+		
Mannitol	none			+		
<i>Gas Formation</i>	none			in all tubes		
Incubation Temp	37° C			37° C		
Strain	1	2	3	1	2	3
Media rotation	S	S	S	Q	Q	Q

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	SMITH AND WALLGREN (GALACTOSE)			SMITH AND WALLGREN DIGEST (SUCROSE)		
MEDIA ROTATION	3	3	3	4	4	4
<i>Morphology</i>						
Size	0.6 × 5μ	0.6 × 4.5μ	0.6 × 5μ	0.3 × 2.4μ	0.3 × 3.5μ	0.4 × 1.5.5μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly and end to end	singly and end to end	singly and end to end	singly bifurcations	singly bifurcations	singly bifurcations
Motility	none	none	none	none	none	none
Gram stain	pos	pos	pos	pos and neg	pos and neg	pos and neg
Remarks	Ends frequently tapering			Showed true branching		
<i>Cultural Characteristics</i>						
Growth	both aerobic and anaerobic			anaerobic		
Milk	coagulates after 48 hr			acid, generally coagulates after 96 hr		
Litmus milk	acid with soft coagulum in about 48 hr			acid, coagulation very slow in occurring		
Butter milk	slight growth in about 72 hr			slight growth in 96 hr		
Tomato milk	slight growth in 48 hr			slight growth in 72 hr		
Whey	some gram negative forms			no growth		
Kulp's to mango	no growth			no growth		
Tomato di- gest	growth, gram pos & neg rods			involution forms		
S and W me- dia	growth			gram pos and neg rods		
S and W di- gest	single rods, good growth			growth		
S and W su- crose	involution forms			abundant growth		
Whey agar	pin point, grayish colonies translucent, fimbriate margins			growth		
Broth	slight turbidity, grayish sediment			no turbidity		
Lactose broth	growth			slight growth		
Glucose broth	gram pos and neg forms			abundant growth		
Acidity	quite low, 5.5 per cent in 5 days			acid		
<i>Fermentation</i>						
Dextrose	++++			++++		
Galactose	++++			++		
Lactose	++++			+		
Maltose	++++			+++		
Sucrose	++++			++++		
Dextrin	none			++		
Mannitol	none			+++		
<i>Gas Formation</i>	none			none		
Incubation Temp	38° C			38° C		
Strains	1	2	3	1	2	3
Media Rotation	10	10	10	11	11	11

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	SMITH AND WALLGREN DIGEST (LACTOSE)			ALPHA WHEY BROTH (LACTOSE)		
MEDIA ROTATION	5	5	5	6	6	6
<i>Morphology</i>						
Size	0.8 × 2.6 μ	0.8 × 2.6 μ	0.8 × 3.5 μ	0.8 × 4.7 μ	0.8 × 4.7 μ	0.8 × 4.7 μ
Form	rods	rods	rods	rods	rods	rods
Arrangement	end to end only	end to end only	end to end only	singly and long chains	singly and long chains	singly and long chains
Motility	none	none	none	none	none	none
Gram stain ing	pos and neg	pos and neg	pos and neg	pos	pos	pos
Remarks	No chains			Fluorescent points at the poles		
<i>Cultural Characteristics</i>						
Growth	aerobic and anaerobic			best growth anaerobic but grows aerobic		
Milk	excellent growth but no coagulation			acid with no clotting		
Litmus milk	acid, no coagulation			acid, no separation of whey but soft creamy curd deposited in time		
Butter milk	involution forms			growth		
Tomato milk	involution forms			some gram neg forms		
Whey	involution forms both gram pos and neg			growth		
Kulp's to mato	no growth			no growth		
Tomato di gest	no growth			involution forms		
S and W me dia	growth			growth		
S and W di gest	growth			growth		
S and W su crose	small rods round on both ends			gram pos and neg rods		
Whey agar	slight growth			small, flat, circular colonies		
Glucose agar	growth			growth		
Broth	no growth in 24 hr			slight growth		
Lactose broth	excellent growth			turbid, growth		
Glucose broth	abundant growth					
Acidity	0.2 per cent in 24 hr as acetic			high		
<i>Fermentation</i>						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	++			+++		
Mannitol						
<i>Gas Formation</i>	none			none		
<i>Incubation Temp</i>	37° C			37° C		
Strains	1	2	3	1	2	3
Media Rotation	12	12	12	13	13	13

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	THIFTA WHEY BROTH (GLUCOSE AGAR)			BUTTER MILK PLUS GLUCOSE TO THIFTA WHEY BROTH		
MEDIA POTATION	7	7	7	8	8	8
<i>Morphology</i>						
Size	$0.3 \times 1.5 \mu$	$0.3 \times 3 \mu$	$0.3 \times 2.5 \mu$	$0.3 \times 1.2 \mu$	$0.3 \times 1.2 \mu$	$0.3 \times 1.2 \mu$
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly	singly	singly	singly and in chains	singly and in chains	singly and in chains
Motility	none	none	none	none	none	none
Gram stain ing	pos	pos	pos	pos	pos	pos
Remarks				Chains of 2 to 6 elements some of the rods showed both gram pos and neg segments		
<i>Cultural Characteristics</i>						
Growth	facultative microbes best growth anaerobic			aerobic and anaerobic		
Milk	coagulates in from 6 to 12 days soft curd no retraction			coagulates in 7 hr at $45^{\circ} \text{C}$		
Litmus milk	acid			acid		
Butter milk	slight growth			growth		
Tomato milk	gram pos and neg forms			growth		
Whey	scanty growth			involution forms		
Kulp's to mango	involution forms			involution forms		
Tomato di gest	involution forms			involution forms		
S and W me dia	involution forms			involution forms		
S and W di gest	gram neg and some gram pos			gram pos and neg		
S and W su crose	gram neg and pos			gram pos and neg		
Whey agar	very scant growth					
Whey glucose	growth			growth		
Glucose agar	growth			growth		
Broth	involution forms			involution forms		
Lactose broth	growth			3 element chains		
Glucose broth	good growth			good growth		
Acidity	very slight			slight		
<i>Fermentation</i>						
Dextrose	+++			+++		
Lactose	+++			+++		
Maltose	+++			+++		
Sucrose	+++					
<i>Gas Formation</i>	none			none		
<i>Incubation Temp</i>	$40^{\circ} \text{C}$			$40^{\circ} \text{C}$		
Strains	1	2	3	1	2	3
Media Rotation	14	14	14	15	15	15

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	GALACTOSE WHEY			GLUCOSE WHEY		
MEDIA ROTATION	9	9	9	10	10	10
<i>Morphology</i>						
Size	115 $\mu$	115 $\mu$	115 $\mu$	115 $\mu$	115 $\mu$	115 $\mu$
Form	circular	circular	circular	circular	circular	circular
Arrangement	pairs	pairs	pairs	singly	singly	singly
Motility	none	none	none	none	none	none
Gram stain	pos	pos	pos	pos	pos	pos
Remarks	In lactose whey pairs and single forms occur			True nitifier		
<i>Cultural Characteristics</i>						
Growth	facultative anaerobes, best growth anaerobic			facultative anaerobes		
Milk	rapid coagulation by fermentation			coagulation by acid (lactic) and not fermentation		
Litmus milk	slowly changes acid			acid		
Butter milk	growth butter milk plus galactose gives a gram pos pair surrounded by gram neg sheath			growth		
Tomato milk	growth			growth		
Whey	growth			growth		
Kulp's to mato	growth			growth		
Tomato digest	growth			growth		
Whey lactose	both single and pairs			circular forms arrange themselves in chains of 3, gram neg sheath surrounds some of these triads, length 3.4 $\mu$		
S and W media	sheathed pairs			growth		
S and W digest	growth			growth		
S and W sucrose	growth			growth		
Broth	growth			growth		
Lactose broth	single and pairs			single, pairs, and triads		
Glucose broth	growth			growth		
Acidity	slight acid reaction			acid reaction		
<i>Fermentation</i>						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	+++			++++		
Sucrose				++++		
Dextrin				++		
Mannitol				+++		
<i>Cas Formation</i>	none in 48 hr			none in 48 hr		
Incubation Temp	37° C			37° C		
Strains	1	2	3	1	2	3
Media Rotation	16	16	16	17	17	17

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	LACTOSE WHEY TO MILK			LACTOSE WHEY		
MEDIA ROTATION	11	11	11	12	12	12
<i>Morphology</i>						
Size	0.5 × 1.2 $\mu$	0.5 × 1.2 $\mu$	0.5 × 2.3 $\mu$	0.8 × 3.6 $\mu$	0.8 × 3.6 $\mu$	0.8 × 3.6 $\mu$
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly and chains	singly and chains	singly and chains	singly and chains	singly and chains	singly and chains
Motility	none	none	none	none	none	none
Gram staining	pos	pos	pos	pos	pos	pos
Remarks						
<i>Cultural Characteristics</i>						
Growth	facultative anaerobes			facultative anaerobes		
Milk	no coagulation but good growth			no coagulation but good growth		
Litmus milk	unchanged			acid but no coagulation		
Butter milk	slight growth			growth		
Tomato milk	growth			no growth		
Whey	involution forms			no growth		
Kulp's tomato	no growth			no growth		
Tomato digest	growth			no growth		
Whey lactose S and W media	reverts back to encicular forms			abundant growth		
Whey lactose S and W media	growth			growth		
S and W digest	no growth			no growth		
S and W sucrose	involution forms			involution forms		
Broth	growth			growth, very slight		
Lactose broth	growth			growth		
Glucose broth	growth			no growth		
Whey agar	growth			no growth		
Lactose agar	growth			small, grayish, viscous colonies		
Acidity	acid reaction			acid reaction		
<i>Fermentation</i>						
Dextrose	+++			++		
Galactose	+++			+++		
Lactose	+++			+++		
Maltose	+++			none		
Sucrose	+++			none		
Dextrin	+++			none		
Mannitol	+++			none		
<i>Gas Formation</i>	none			in dextrose and lactose		
<i>Incubation Temp</i>	37° C			40° C		
Strains	1	2	3	1	2	3
Media Rotation	18 or 1	18 or 1	18 or 1	2	2	2

TABLE III (Continued)

STRAINS	4	5	6	4	5	6
MEDIA	GALACTOSE WHEY			DELT A WHEY BROTH (MALTOSE)		
MEDIA ROTATION	13	13	13	14	14	14
Morphology						
Size	1 × 3 4 $\mu$	1 × 3 4 $\mu$	1 × 3 4 $\mu$	1 × 4 6 $\mu$	1 × 4 6 $\mu$	1 × 4 6 $\mu$
Form	rods	rods	rods	rods	rods	rods
Arrangement	singly	singly	singly	single and chains	single and chains	single and chains
Motility	none	none	none	none	none	none
Gram stain						
ing	pos	pos	pos	pos and neg	pos and neg	pos and neg
Remarks				chains of 2 to 3 elements showed both gram pos and neg segments		
<i>Cultural Characteristics</i>						
Growth	facultative anaerobes			facultative anaerobes		
Milk	good aerobic growths coagulates into a firm clot, good growth, no peptonization			good aerobic growths coagulation		
Litmus milk	acid			acid with coagulation		
Butter milk	growth			growth		
Tomato milk	growth			growth		
Whey	growth			growth		
Kulp's to mato	involution forms			involution forms		
Tomato digest	no growth			growth		
Whey lactose	growth			coccoid shaped forms		
S and W medium	growth			involution forms		
S and W digest	slight growth			slight growth		
S and W sucrose	no growth			growth		
Broth				slight turbidity		
Lactose broth	slight growth			slight growth		
Glucose broth	growth			slight growth		
Whey agar	small, transparent colonies					
Acidity	4 per cent in dextrose medium			2.5 per cent in dextrose medium		
<i>Fermentation</i>						
Dextrose	++++			++++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	++++			++++		
Sucrose	++++			++++		
Mannitol				++++		
<i>Gas Formation</i>	none			none		
Incubation Temp	17° C and 35° C			37° 34° C		
Strains	1	2	3	1	2	3
Media Rotation	3	3	3	4	4	4



TABLE III (Continued)

STAINS	4	5	6	4	5	6
MEDIA	WHEY			KULP'S TOMATO WHEY		
MEDIA ROTATION	15	15	15	16	16	16
<i>Morphology</i>						
Size	1 x 3 16 $\mu$	1 x 3 16 $\mu$	1 x 3 16 $\mu$	2 x 4 20 $\mu$	2 x 4 20 $\mu$	2 x 4 20 $\mu$
Form	rods	rods	rods	rods	rods	rods
Arrangement	chains	chains	chains	chains	chains	chains
Motility	none	none	none	none	none	none
Gram stain	pos	pos	pos	pos and neg	pos and neg	pos and neg
Remarks	Chains with no true branching Often over 200 $\mu$ in length			Chains with 2 to 10 elements The organisms did not attract casein		
<i>Cultural Characteristics</i>						
Growth	aerobic			facultative anaerobes, best growth anaerobic		
Milk	coagulates with a firm clot and little serum			acid and coagulation in from 10 to 15 hr		
Litmus milk	acid with curd			acid with soft, creamy curd in 24 hr		
Butter milk	abundant growth			abundant growth		
Tomato milk	growth			involution forms		
Whey	abundant growth			turbid, grayish white sediment		
Kulp's to	growth			abundant growth		
mato						
Tomato digest	involution forms			growth		
S and W medium	involution forms			growth		
S and W digest	involution forms			rods divide into gram pos and gram neg		
S and W sucrose	no growth			revert to coccoid shape		
Whey agar	small, white colonies about 1 mm in dia			circular to irregular, grayish white, filamentous colonies		
Acidity	15.25 per cent			27.4 per cent in milk		
<i>Fermentation</i>						
Glucose	+++			+++		
Galactose	++++			++++		
Lactose	++++			++++		
Maltose	+++			++++		
Mannitol				++++		
<i>Gas Formation</i>	none			none		
Incubation Temp	37° C			40° C		
Strains	1	2	3	1	2	3
Media Rotation	5	5	5	6	6	6

TABLE III (Concluded)

STRAINS	4	5	6	4	5	6
MEDIA	TOMATO NUTRIENT BROTH			TOMATO DIGEST BROTH		
MEDIA ROTATION	17	17	17	18 or 1	18 or 1	18 or 1
<i>Morphology</i>						
Size	$0.5 \times 7\mu$	$0.5 \times 7\mu$	$0.5 \times 7\mu$	$1 \times 8.5\mu$	$1 \times 8.5\mu$	$1 \times 8.5\mu$
Form	rods	rods	rods	rods	rods	rods
Arrangement	single and chains	single and chains	single and chains	pairs and chains	pairs and chains	pairs and chains
Motility	none	none	none	none	none	none
Gram stain						
ing	pos and neg	pos and neg	pos and neg	pos	pos	pos
Remarks	In liquid media a space appears between elements, rods often appear in parallel			Some strain poorly		
<i>Cultural Characteristics</i>						
Growth	facultative anaerobes			facultative anaerobes		
Milk	coagulates in 6 hr and produces 0.3 per cent acid $37^{\circ}\text{C}$			acid with coagulation and no digestion of clot		
Litmus milk	acid and no digestion of clot			acid with coagulation		
Butter milk	slight growth			slight growth		
Whey	slight growth			no growth		
Kulps to	gram pos and neg rods			no growth		
mato						
Tomato di	growth			gram neg and pos forms		
gest						
S and W me	growth			growth		
dia						
S and W di	involution forms			growth		
gest						
S and W su	involution forms			slight growth		
crose						
Whey agar	slight growth			no growth		
Nutrient	no growth			no growth		
agar						
Lactose agar	growth					
Lactose broth	growth			slight growth		
Glucose broth	growth			growth		
Acidity	0.3 per cent in 24 hr as lactic			1 per cent in milk in 24 hr		
<i>Fermentation</i>						
Glucose	+++			+++		
Galactose				+++		
Lactose	+++			+++		
Maltose	+++			++		
Sucrose						
Mannitol				none		
<i>Gas Formation</i>	none			none		
Incubation						
Temp	$37^{\circ}\text{C}$			$37^{\circ}\text{C}$		
Strains	1	2	3	1	2	3
Media Rotation	7	7	7	8	8	8

characteristic colonies (2 to 4 mm dia and 0.7 to 1 mm dia) were identical in morphology but differed culturally in so far as the larger colonies were surface growths and aerobic, while the smaller colonies were deep growers and anaerobic.

The various purified strains were then successively grown on a series of different media. The morphology of the organism was checked every twenty-four hours by staining with Kopeloff's modified gram stain<sup>25</sup> and examining under the microscope. At various intervals depending upon a change in morphology of the organism the cultural characteristics were determined. The results are recorded in Table III. Strains 4, 5 and 6 were rotated successively on tomato digest containing dextrose, tomato digest containing lactose, S and W medium containing galactose, predigested S and W medium containing sucrose, predigested S and W medium containing lactose, lactose whey broth, glucose whey broth, buttermilk fortified with glucose, glucose whey broth, galactose whey, glucose whey, lactose whey, milk, lactose whey, galactose whey, maltose whey broth, whey, Kulp's tomato whey, tomato nutrient broth and tomato dextrose digest medium. Strains 1, 2, and 3 were transferred to whey, buttermilk fortified with galactose, lactose whey, milk, glucose whey, and then rotated as in strains 4, 5, and 6 starting with glucose whey. The method of preparation of these various culture media is described in a subsequent paper.

The number of times an organism was transferred to the same media, the length of time of incubation, and the point at which a new medium was employed, depended upon the morphologic characteristics of the organism when stained and viewed under the microscope. A considerably longer time was required to transform the circular into the rod shape than the reverse.

The morphologic changes and cultural characteristics are recorded in Table III. A review of the literature on the morphology and cultural characteristics of the more definitely established members of the lactobacilli group<sup>20, 21, 22, 23</sup> describe *Lactobacillus lactisacidi* (Leichmann) as a rod shaped organism varying from 0.8 to 1.2 micron in length and 0.5 micron in breadth. They appear singly and in chains. The ends of the bacillus are square and they are gram-positive. Optimum temperature 40° C. Their natural habitat is milk. They are microaerophilic and grow in milk and broth, and form soft grayish, transparent filiform growths on agar slants. Litmus milk is unchanged. They do not form indol nor reduce nitrates. Acid is produced. Acid fermentation was reported in dextrose, maltose, fructose, lactose and dextrin without the formation of gas. Arabinose and trehalose were not fermented.

*Lactobacillus helveticus* (*Lactobacillus casei*) are described as rod shaped organisms, 0.7 to 0.9 micron in breadth and 2 to 6 microns in length. They appear singly and in chains, nonmotile, and gram-positive. Optimum temperature 40 to 42° C. Their natural habitat is sour milk and cheese. They are microaerophilic, do not coagulate milk, and show no growth in broth, whey, gelatin, whey agar, nor gelatin. Deep growth occurs in lactose agar, as well as in small, grayish, and viscid colonies. Litmus milk becomes acid without coagulation. They do not form indol nor reduce nitrates. Acid fermentation with the production of gas occurred in lactose and dextrose. Maltose and mannitol were not fermented.

*Koichen bacillus* of Leunssen and Kuhn, are described as rod shaped organ

isms, nonmotile, and gram-positive. They stain in Neisser and alkaline methylene blue. Optimum temperature 37 to 45° C. Their natural habitat is buttermilk. Good growth occurs in carbohydrate media (milk plus carbohydrates) with the coagulation of the milk into a firm clot at a temperature of 37° C. There is no evident peptonization. Indol is not formed, nor nitrates reduced. Acid fermentation occurs in carbohydrate medium without the production of gas.

*Lactobacillus pabuliacidus* (Bergey) are described as rod shaped organisms occurring singly and in long chains. They are nonmotile and gram-positive. Optimum temperature 34 to 40° C. Their natural habitat is beet mash and cheese. They are aerobic, coagulate milk, and produce a slight turbidity in broth. A slightly arborescent growth occurs in gelatin stab, and small, transparent colonies on agar. Four per cent acidity is produced in dextrose. Acid fermentation, without the production of gas, occurs in lactose, sucrose, maltose, mannitol, and glycerol. Raffinose is not fermented. Litmus milk becomes acid without coagulation.

*Bacterium mazum* (Weigmann, Gruber, and Huss) are described as gram-positive rods, 1 to 1.1 microns in breadth and 2.7 to 2.1 microns in length. They form chains with no true branching, and are nonmotile. The ends of the rods are swollen, and cells with true branching are reported in whey. Involution forms occur in the shape of rods 2.10 microns in length. They stain with carbolfuchsin, and their optimum temperature is 37° C. They are aerobic, coagulate milk with a firm clot and little serum, and grow abundantly in whey. Small, white colonies about 1 mm. in dia. occur in whey agar. They produce acid.

*Lactobacillus bulgaricus* are described as rod shaped organisms, 1 to 2 microns in length (Bergey), 2 to 20 microns in length (Foid), and forming chains. They are gram-positive in young cultures and gram-negative in old ones. They are nonmotile, stain with all ordinary stains, and form chains of 2 to 10 elements in length. They are facultative anaerobes. They grow rapidly in milk, coagulation occurring in from ten to twenty-four hours at 37° C. and an acidity of from 2.7 to 3.7 per cent is produced, i. e., 32.5 gm. lactic acid per liter of milk in ten hours while other members produce about 10 grams per liter in the same length of time. Litmus milk becomes acid in twenty-four hours with the production of a soft, creamy curd. They produce a turbidity in whey with a grayish white sediment, and circular to irregular, grayish-white, filamentous colonies on whey agar. The agar colonies are small, thick, lenticular, umbilicated and 0.1 mm. in diameter. Acid fermentation, without the production of gas, occurs in dextrose, lactose, galactose, fructose, and mannitol. Rhamnose, dulcitate, and sorbite are not fermented. This organism does not attack casein, has its natural habitat in yoghurt and buttermilk, and its optimum temperature is 45° C.

*Streptobacillus lebensis* (Rist and Khoury) are described as rod shaped organisms, nonmotile, 0.5 micron in breadth, and 6 to 8 microns in length. They occur singly and in chains up to 18 microns in length. They have been reported found in parallel bundles and in liquid media a space appears between the elements of the chain. They are gram-positive in young cultures, but the dead organisms are gram-negative. Optimum temperature 37 to 40° C. Their natural habitat is fermented milk. They grow aerobic and anaerobically and coagulate

milk in six hours at 37° C with the production of 0.26 per cent acid, calculated as lactic. A true rennin ferment is produced with no digestion of the clot. A slight growth is obtained in whey and better growths on glucose and lactose agar. Gas is not produced on fermentation. They stain with carbolfuchsin.

*Lactobacillus Boas-Oppley* (Boas and Oppley) are described as rod shaped organisms occurring in pairs and chains, nonmotile, and gram-positive. According to Ford these rods are 1 micron in breadth and from 2 to 10 microns in length while Bergey finds them 6 to 8 microns in length. Red metachromatic granules are obtained with methylene blue. Optimum temperature 40° C. Their natural habitat is the gastric contents, especially gastric carcinoma. Although microaerophilic, they grow best anaerobically. Best growths are obtained on acid media, broths plus glucose or beer wort, and media containing gastric cancer extract. Litmus milk is turned acid with coagulation, and small, gray, granular colonies appear on milk agar. One per cent acidity is produced in milk. Nitrates are not reduced. Acid fermentation without the production of gas occurs in dextrose and lactose. Mannitol is not fermented.

*Lactobacillus acidophilus-aerogenes* (Torrey and Rahe) are described as rod shaped organisms, gram-positive, occurring singly and in chains up to 40 microns in length with occasional branching or Y forms. The rods are 0.8 micron in breadth and from 1.5 to 11 microns in length (Bergey gives 5 microns as the maximum length). They are nonmotile and stain with Loeffler's alkaline methylene blue. Optimum temperature 37° C. Their natural habitat is the intestinal tract. They can grow aerobically and anaerobically, and clot milk in seventy-two hours with the partial separation of whey. Acid coagulation occurs in litmus milk. Growth occurs on gelatin and agar but best on glucose oleate agar or 5 per cent acetic acid glucose. Nitrates are not reduced. Ten per cent acidity is produced in three days. Acid fermentation occurs in dextrose, lactose, sucrose, and raffinose, with the production of gas. Arabinose is not fermented.

*Lactobacillus acidophilus* are described as slender, gram-positive rods occurring singly and in short chains usually of 2 to 3 elements. They are from 4 to 5 microns in length and the ends of the rods are found to be frequently tapered. They are nonmotile and the metachromatic granules are stained by Neisser's method. Optimum temperature 37 to 45° C. Their natural habitat is the human infant intestine. They grow both aerobically and anaerobically. Milk is generally reported to be coagulated after forty-eight hours and litmus milk becomes acid with the production of a soft coagulum in the same length of time. They produce a slight turbidity in broth with a gray sediment. Gray, fimbriate colonies slowly develop on gelatin and very scanty growth occurs in the gelatin stab. The colonies on agar are thin, irregular, and translucent with fimbriate margins, and the slant shows a filiform, smooth, and slimy growth. Good growth occurs on glucose agar. Indol is not formed. This organism produces a low acidity, 5.5 per cent in five days in dextrose broth. Acid fermentation without the formation of gas occurs in dextrose, lactose, maltose, raffinose, and sucrose. Mannitol and dextrin are not fermented.

*Lactobacillus bifidus communis* (Tissier) are described as rod shaped organisms, 0.3 to 0.6 micron in breadth and 1.5 to 6 microns in length. They occur singly, showing bifurcations and true branching. The young cultures are gram-

positive, while the old cultures and the dead organisms are gram-negative. They are stained with basic anilin dyes. Optimum temperature  $37^{\circ}\text{C}$ . Their natural habitat is the intestine. They are strict anaerobes and grow abundantly in glucose broth. Acid coagulation of milk takes place in time, and litmus milk may or may not be coagulated. Poor growth occurs in gelatin. The colonies on glucose agar are deep seated and have an average diameter of 2 mm with a maximum of 4 mm. Acid fermentation occurs in dextrose, lactose, maltose, sucrose, raffinose, and mannitol.

*Lactobacillus lebensis* (Rist and Khouby) are described as rod shaped organisms, 2 to 6 microns in length and arranged end to end without the formation of chains. The young cultures are gram-positive, while the old cultures and dead organisms are gram-negative. They are nonmotile and appear granular (similar to the tubercle bacilli) in whey. Their natural habitat is milk. They grow both aerobically and anaerobically and produce about 0.2 per cent acid (as lactic) in twenty-four hours. Excellent growth occurs in milk without coagulation, and litmus milk is turned acid. Good growth occurs in lactose or glucose broth, whey, glucose or lactose agar, and lactose whey.

*Lactobacillus caucasicus* (Kern, Beijerinck) are described as nonmotile, gram-positive, rods, 0.8 micron in breadth and 3.2 to 8.0 microns in length. They occur singly and in long chains and have glistening points at the poles. Optimum temperature  $37$  to  $40^{\circ}\text{C}$ . They grow both aerobically and anaerobically, the growth often being delayed two to three weeks. Milk becomes acid without clotting. Litmus milk develops 1.2 to 1.6 per cent acidity (as lactic) and a soft creamy curd. Lactose broth becomes turbid, and a growth occurs in both whey agar and whey gelatin. Small, flat, grayish, circular, undulated surface colonies, and deep, fimbriate colonies occur in agar. The growth on agar slants is scanty, while a grayish growth occurs in agar stabs. Indol is not formed, nor are nitrates reduced. Acid fermentation without gas production occurs in dextrose and lactose. Mannitol is not fermented.

*Bacillus exilis* (Tissier) are described as nonmotile, gram-positive rod shaped organisms, whose natural habitat is the stools of infants. They stain with dilute fuchsin, gentian violet, and thionin. Their optimum temperature lies between  $37$  and  $40^{\circ}\text{C}$ . They are facultative anaerobes and do not grow on acid media. They coagulate milk in eight to ten days with a soft curd and no retraction. Litmus milk is turned acid. A deep growth occurs in glucose agar at  $20$  to  $37^{\circ}\text{C}$ , and they produce no gas on fermentation.

*Streptobacillus C* (Grigoroff) are described as nonmotile, gram-positive, rod shaped organisms. Their natural habitat is buttermilk. Optimum temperature  $37$  to  $45^{\circ}\text{C}$ . They are facultative anaerobes, coagulate milk in six hours at  $45^{\circ}\text{C}$ , and produce acid in litmus milk. They produce buttermilk at  $37^{\circ}\text{C}$  in from twelve to twenty-four hours. They are true denitrifiers and do not form indol. Acid fermentation occurs in lactose, as well as in lactose broth.

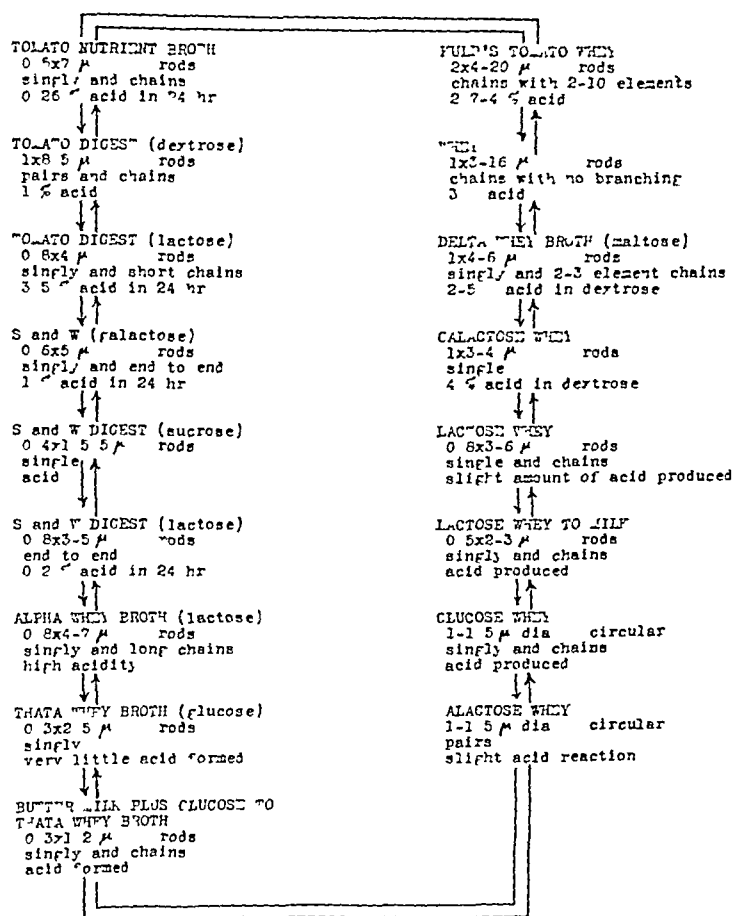
*Diplococcus lebensis* (Rist and Khouby) are described as nonmotile, gram-positive, circular organisms, which always occur in pairs. These organisms take the usual stains and do not form spores. Growth occurs in glucose broth, whey, and lactose agar. A rapid coagulation occurs in milk and when it is kept alkaline or neutral with sodium carbonate, a rennin-like ferment is produced.

*Micrococcus B* (Gigoriotti) are described as nonmotile, gram-positive, circular organisms, which always occur singly. Their natural habitat is buttermilk. They grow both aerobically and anaerobically. Buttermilk is produced in less than twenty-four hours at 37° C. Milk is coagulated by the production of lactic acid and not by fermentation. Good growth occurs in glucose broth. These organisms are true nitrifiers and give a positive nitroindol reaction. They are acid producing organisms. Acid fermentation occurs in fructose, rhamnose, maltose, saccharose, mannite, and glycerin. Dulcitate and sorbite are not fermented.

## LACTOBACILLI CYCLE

On comparison of the morphologic and cultural characteristics of Table III with those of the members of the lactobacilli group described in literature, a striking similarity between the various bacilli was observed. When Table III is arranged in the form of a cycle, Table IV is obtained. A critical study of Table IV brought out this peculiarity, that the cycle naturally divides itself into two

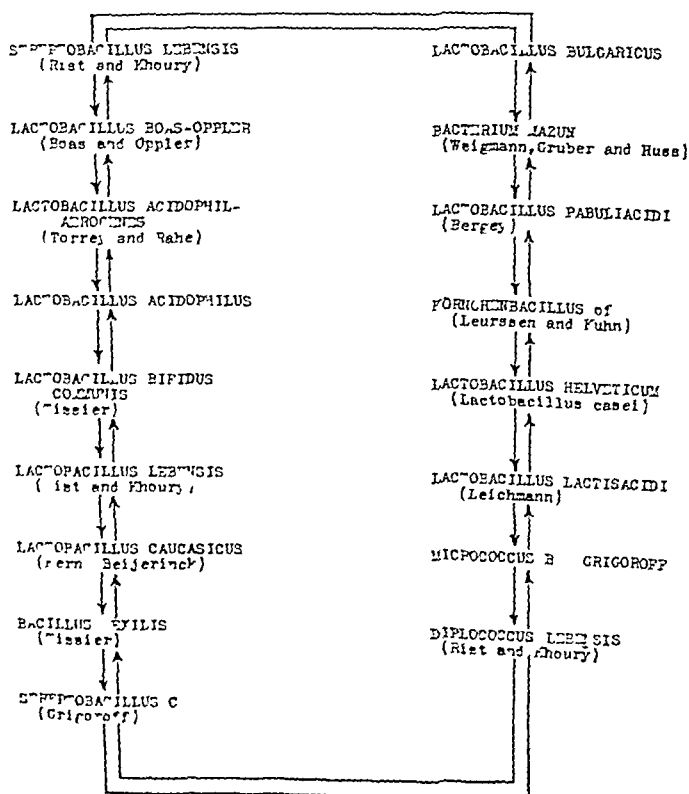
TABLE IV  
LACTOBACILLUS CYCLE FROM PURIFIED STRAINS



parts The left-hand, or anaerobic part, in which the organisms were difficult to culture required a specialized media based on the chemical and physical constitution of the contents of the intestine at different life periods, and grew best under anaerobic conditions at body temperature The right-hand part consisted chiefly of facultative anaerobes cultured on milk and whey medium, and grew best under aerobic conditions This is not a clear cut division, since some organisms, which are located at the top and bottom of the cycle illustrated in Table IV, grew equally well aerobically and anaerobically The medium, in this case, giving the best results were of a combined nature

Upon derivation of a cycle from such lactobacilli organisms as were found to be sufficiently described in literature, and arranging them according to the locations indicated in Table IV, Table V was obtained This table showed that if we were to start with a pure culture of the *Bacillus acidophilus*, or in fact any other lactobacilli, we could obtain by a process of successive selective culturing any other desired member of this group At least an organism having the morphologic and cultural characteristics of the desired bacillus could be obtained These changes were regulated by the food supply (nature of the medium), the  $P_H$  value of the medium, and the temperature of incubation

TABLE V  
LACTOBACILLI CYCLE  
ACCORDING TO THE NOMENCLATURE FOUND IN LITERATURE





## THE NATURE OF THE MORPHOLOGIC CHANGE IN THE BACILLI

Two processes are involved, one of synthesis and the other of a destructive or degenerative nature. In the latter case the changes in the bacillus occurred quickly and readily, and ultimately terminated in a coccoid shaped end product. The process of synthesis required much skill in the rotation of medium, its selection and the time of incubation. Starting with a chain, we observed a gram-negative section appearing between the elements of which it was composed. A separation then occurred at these junctions with no reduction in the length of the component rods. Gram-negative and gram-positive segments then appeared in the rods. Usually the gram-negative section was observed between two gram-positive components. In some cases the rod showed a division in the middle, half staining gram-positively and half gram-negatively. Division into gram-positive and gram-negative rods now occurred. The gram-negative rods



Fig 1 —Involution and synthesis of the Lactobacilli

next changed over into gram-positive rods of the same size. These smaller rods again split as described above. When a length of from 1.5 to 2 microns was obtained, the rod slightly bulged out at a short distance from both ends, and stained gram-positively. These bulges developed into two circular elements contained at either end of the rod. On staining, the circular portion became gram-positive, and the rest of the rod, gram-negative. A few circular forms were observed connected by a restriction through their centers, the whole staining gram-positively, but this occurred only in rare cases. Separation next occurred into circular pairs which took a gram-positive stain. The pairs were then observed to break up. The circular forms next arranged themselves into short chains generally consisting of three elements and stained gram-positively. A fusion then occurred, which on staining, showed three gram-positive circles contained within a gram-negative rod. The gram-positive circles then faded out leaving a gram-negative rod, which then changed over into a gram-positive rod. We were unable to determine at this point whether all the gram-negative rods changed over into gram-positive rods and then a specialized number changed back again.

into gram-negative forms or only a part of them changed over into gram-positive rods. In every case, however, mixtures of gram-positive and gram-negative rods were obtained. The next step consisted in the fusion of the gram-negative and gram-positive rods into a single rod consisting of a gram-negative central portion and gram-positive ends. Three of the original rods entered into this fusion. This rod then changed over into a gram-positive rod of approximately the same length as the rods which entered into its synthesis. The size of the rod was then increased by the fusion of two gram-positive rods lying end to end, although, in some cases, fusion into a single rod occurred from chains of four

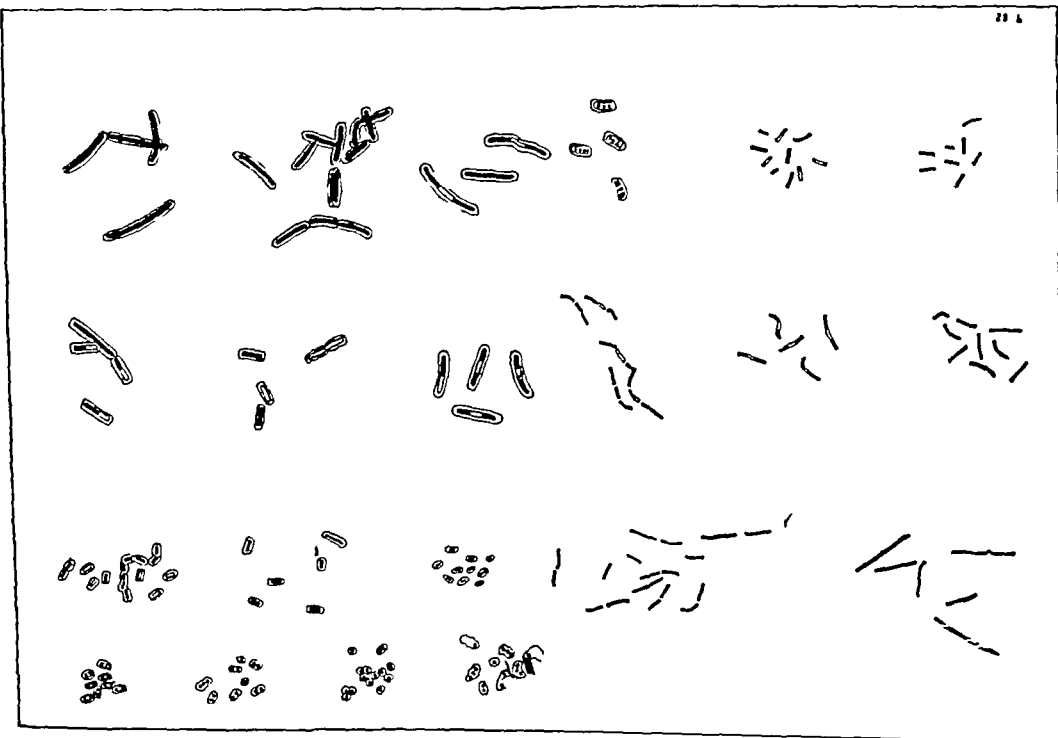


Fig. 2—Morphologic changes in the culture of the lactobacilli. Camera lucida drawing 1650X

gram positive rods. This process is diagrammatically illustrated in Fig. 1. The scale of the diagram is exaggerated so as to bring out the details of structure. Since microphotographs did not bring out the gram-negative elements nor the capsulated forms, camera lucida drawings of the process were made and are reproduced in Fig. 2.

#### DISCUSSION

The results obtained in this laboratory indicate that any member of the lactobacilli group may be changed into or caused to assume, the morphology and cultural characteristics of any other bacillus in this group through a successive culturing on selective media. The incubation period varied from a twenty-four hour culture in the case of the reduction in size of the rod, to one

hundred and ten hours. The higher figures were always characteristic of the synthesis of the rod and particularly from the circular forms. This process of synthesis is in agreement with the combined observations of Hadley, Solarino, Kendall, and Haudurov.

The impracticability of the implantation of the *Bacillus acidophilus* now becomes apparent since it would revert over to a type acclimated to the surrounding media. This would be particularly true when treatment with the artificial media ceases, i. e., supplementary treatment with lactose, agar, bran, paraffin, mucilaginous products obtained from seeds, etc. To continue the supplementary treatment required by *acidophilus* therapy over a long period of time would certainly produce a pathologic condition of the intermediate metabolism of the digestive system (including the enzymic and hormonal systems). To discontinue this supplementary treatment would soon result in a reversion of the introduced *lactobacillus* to a type further down the cycle, whose natural habitat is the normal adult intestine and is capable of utilizing the amino acids for food. Whether or not they digest the amino acids by deamination, decarboxylation, or both, is at the present time uncertain, but under pathologic conditions (chronic constipation, etc.) they most certainly will aid in the production of toxic substances. We found that the *Bacillus acidophilus* grew best on an artificial media approximating as closely as possible the conditions found in the intestines when food passes through the duodenum of a breast-fed infant. After the infant is weaned, the intestinal flora changes rapidly and putrefactive types appear. It is our belief that the *Bacillus acidophilus* does not disappear from the adult intestine but will be found in relatively the same numbers in a form resembling the coccus or the diplococcus, and will have the cultural characteristics of that organism. Since the intestinal tract of a newborn infant is sterile as well as the intestinal tract of numerous polar animals, and since it has been demonstrated that chicks and flies grown under sterile conditions function normally, we believe that the presence of bacteria of any description in the human intestinal tract is unnecessary and to add them is certainly unwise. For no matter what type of nonpathogenic organism may be added, they will revert to an organism capable of producing toxins, or interfering with the normal functioning of the body, according to its food supply.

#### SUMMARY

- 1 The morphology and cultural characteristics of the *Bacillus acidophilus* depends upon the nature of the food supply, the  $P_H$  value of the medium and the presence of small quantities of inorganic metals, such as zinc, copper, iron, etc.

- 2 Any member of the *lactobacilli* group may be converted into, or caused to assume, the morphologic and cultural characteristics of any other member.

- 3 The members of the *lactobacilli* group may be arranged in a cycle according to their morphology and cultural characteristics.

- 4 Any member of this cycle in assuming the morphology and cultural characteristics of any other member passes successively through the characterization of all intervening members in the cycle.

5 The cycle is capable of division into two natural parts, one composed of members whose usual habitat is intestinal, and the other contains the nonintestinal members

6 In the case of adverse conditions, if the organism survives, most of the nonintestinal and practically all of the intestinal forms assume the coccoid shape

7 The change in morphology extends over a considerable period of time, even under the most favorable conditions This is particularly true in the synthesis of the rod shaped bacilli

8 The rods pass through a definite process in their synthesis and splitting, in which an interplay of gram-negative and gram-positive forms occur

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## A STUDY OF THE LACTOBACILLI MEDIA EMPLOYED\*

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IN THE first paper of this series, it was shown that the members of the lactobacilli group may be arranged in a cycle according to the nature of the food supply and the conditions of growth (aerobic, anaerobic, facultative anaerobic,  $P_H$  value and incubation temperature) which are characteristic for each bacillus. It was further shown that any member of the lactobacilli group may be changed into, or caused to assume the morphology and cultural characteristics of any other bacillus in this cycle through a successive culturing on selective media. The object of this paper is to describe these various media.

The media employed to obtain the characteristic morphology of the lactobacilli located on the nonintestinal side of the cycle, were found described in literature. Whey and whey agar, prepared according to Rettger and Horton,<sup>1</sup> to which the variously indicated sugars were added, was employed. Kulp's tomato medium<sup>2</sup> was also used.

In determining the medium to be employed for the growth of the organisms listed on the intestinal side of the cycle, a careful study of the habitat of the individually isolated and recorded members of the lactobacilli group was made. The source of most of these bacilli was found to be the intestinal canal. Chemical analyses of the intestinal contents during digestion were studied and the following media developed.

MEDIA	ALPHA WHEY BROTH	DELTA WHEY BROTH	THETA WHEY BROTH
	(PER CENT)	(PER CENT)	(PER CENT)
Whey	94.1	94.1	94.1
Sugar	0.4 (lactose)	0.4 (maltose)	0.4 (glucose)
Casein	1.4	1.4	1.4
Albumin	1.95	1.95	1.95
Pepsin	1.95	1.95	1.95
Sodium citrate	0.2	0.2	0.2

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Tomato nutrient broth was prepared by dissolving 8 grams of dehydrated nutrient broth (Difco) and 8 grams of sodium chloride in 500 c c of water. Then 200 c c of tomato juice were added and the whole was made up to 1000 c c. It was then filtered through cheesecloth, adjusted to a  $P_H$  of 5.2, and sterilized in the autoclave.

## LACTOBACILLUS ACIDOPHILUS MEDIA

The natural habitat of the *Bacillus acidophilus* is the intestinal tract of an infant during the early period of lactation. Many investigators have reported the intestinal tract of a newborn babe free from bacteria, but shortly after birth a bacterial flora develops which contains organisms of the lactic acid group. After the infant is weaned the intestinal flora changes, and putrefactive types of bacteria appear. Thus, the most natural food supply for the *bacillus acidophilus* would be digested human milk at a  $P_H$  of the intestine of an infant during the early stages of lactation. This would require a medium similar in composition to human colostrum.

A chemical study of the early milk of lactation (colostrum) showed some surprising differences in composition when compared to normal human milk. The outstanding differences were the higher value for total solids and total proteins and the relatively lower lactose value. An analysis of colostrum compared to normal human milk is given in Table I. Another important factor in the composition of human colostrum is the relatively large amount of zinc ion. Zinc salts have been reported to coagulate milk more readily than any other kind of inorganic salt (Andreitchewa<sup>3</sup>). They have a peroxydase function but cannot function as an oxydase or catalase. They exhibit an unfavorable effect upon the action of protolytic ferments (pepsin and trypsin). Zinc opposes the action of catalase as shown by Zlataroff<sup>4</sup>. Contrary to general belief, zinc is not toxic in concentrations up to 0.5 parts per 100 (Heller and Burke)<sup>5</sup> and previously reported poisoning were due to impurities (Batchelor, Fehnel, Drinker, and Thompson<sup>6</sup>).

TABLE I  
ANALYSIS OF MILK

KIND	HUMAN			COW	
	NORMAL	NORMAL AVE	COLOSTRUM	NORMAL	COLOSTRUM
SOURCE	1	2	3	4	5
	(PER CENT)	(PER CENT)	(PER CENT)	(PER CENT)	(PER CENT)
Specific gravity	1.0298	1.0313	1.0330	1.0313	1.042
Water	87.58	88.20	82.05	87.27	75.07
Fat	3.74	3.33	4.40	3.68	3.97
Lactose	6.37	6.80	2.64	4.94	2.28
Total Proteins	2.01	1.50	9.96	3.39	17.18
Casein	0.80	1.00	3.11	2.88	4.19
Albumin	1.21		6.85	0.51	12.99
Total Ash	0.30	0.20	0.95	0.72	1.53
Total Solids	12.42	11.80	17.95	12.73	24.93

(1) Heineman Milk. (2) Average of 10 analyses made by one of the authors. (3) Single analysis made by one of the authors. Calculations based on 2 and 5 gave a result in very close agreement with 3. (4) Heineman Milk. (5) Heineman Milk.

From a consideration of the data we developed three media for the culturing of the lactobacilli. It was found necessary to add 0.02 per cent of zinc in the form of the chloride (0.04 per cent) to the digested artificial media when culturing a lactobacillus which would give the morphological and cultural characteristics of *B. acidophilus*. Whether the zinc aided in the coagulation and digestion by stimulating the action of the peroxidases or by limiting the production of the amino acids through its inhibiting action on the proteolytic ferments, pepsin and trypsin, is not known. Zinc will accelerate the coagulation of a partially decalcified milk by rennin.

The medium is adjusted to  $P_H$  7 and autoclaved.

#### S AND W DIGESTED MEDIUM

S and W medium was digested with hydrochloric acid ( $P_H$  2) for 4 hours. Adjusted to  $P_H$  8 with NaOH and then digested for 4 hours with pancreatin. Adjusted to  $P_H$  7 and autoclaved.

#### S AND W MEDIA

	HUMAN COLOSTRUM	FAT AND H <sub>2</sub> O FREE BASIS	WET BASIS	500 C.C. OF MEDIA
	(PER CENT)	(PER CENT)	(PER CENT)	GRAMS
Lactose	5.44	45.15	8.11	40.6
Casein	1.45	12.03	2.16	10.8
Albumin	3.27	27.13	4.87	24.4
Peptone	1.50	12.45	2.24	11.2
Fat	5.90			
Citric Acid		0.82	0.15	0.5
Lactic Acid		0.95	0.17	0.9
Zinc (as ZnCl <sub>2</sub> )				0.04
Zn		0.01	0.002	
NH <sub>4</sub> PO <sub>4</sub>		0.80	0.15	0.8
CaCl <sub>2</sub>		0.65	0.12	0.6
Ash	0.39			
Total Solids	17.95	99.99	17.972	
Water	82.05		82.028	500 c.c.

#### S AND W SUCROSE MEDIUM

Sucrose was substituted for lactose in S and W medium and the digestion carried out as indicated under S and W digested medium.

When correctly prepared the S and W medium after sterilization, had the consistency and color of chyme and was odorless. Amino acid splitting organisms produced a black color and putrefactive odor in twenty-four to forty-eight hours at an incubation temperature of 37° C.

#### TOMATO DIGEST MEDIUM

Lactose or glucose	13.51 per cent
Casein	3.53
Albumin	8.11
Peptone	3.73
Water	71.12

The above mixture is hydrolyzed with 0.4 per cent hydrochloric acid for 4 hours at 37° C, and adjusted to  $P_H$  7. It was then digested with pancreatin.

for 4 hours at 37° C To 60 parts of the above digested medium 40 parts of the following solution were added and the whole adjusted to P<sub>H</sub> 7 and autoclaved

NaCl	0.9 grams
NaH <sub>2</sub> PO <sub>4</sub>	0.15
CaCl <sub>2</sub>	0.12
ZnCl <sub>2</sub>	0.01
Tomato Juice (filtered)	38.8

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## THE BLOOD PRESSURE LOWERING EFFECT OF BISMUTH SUBNITRATE BY MOUTH IN NORMAL AND INCREASED ARTERIAL TENSION\*

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THE nitrite effects of enteric bismuth subnitrate were first manifested clinically in toxic symptoms which followed its early application in x-ray visualization of the gastrointestinal tract. Sailer and Pancoast<sup>1</sup> presented several occurrences of such intoxication after oral administration. Rautenberg<sup>2</sup> described the symptomatology after enema in an almost fatal case. Prior<sup>3</sup> reported it in an infant after over-dosage. Further instances were adduced by Meyer,<sup>9</sup> Zabel,<sup>4</sup> Phillips,<sup>5</sup> and Stieglitz.<sup>15</sup>

Chemico-pathologic findings in fatalities demonstrated the intermediation of nitrite and located the site of its production. Hildebrand<sup>6</sup> referred to two infant deaths after anal injection. In Benneke and Hofmann's<sup>7</sup> premature infant, necropsy showed bismuth in the colon and rectum, a brownish discoloration of the tissues, and methemoglobinemia. Bohme's<sup>8</sup> infant revealed the presence of nitrite in the blood and pericardial fluid, and an absence of bismuth from the blood and liver. In Meyer's<sup>9</sup> adult with intestinal tuberculosis nitrite was present in the feces between two strictures of the small intestine. Nowak and Gutig<sup>10</sup> reported the death of an adult after enema. In Zadek's<sup>11</sup> case of gastric carcinoma with achlorhydria, bismuth was distributed throughout the intestinal canal, nitrite was present in the content of all segments, but especially of the lower portions, of the small intestine, and nitrate was demonstrable in the large intestine. Thus bismuth was present in the colon,<sup>3, 7-11</sup> nitrite was produced in the large intestine, and under abnormal conditions in the small intestine,<sup>9, 11</sup> and circulated in the blood,<sup>8, 9</sup> and methemoglobin developed in the blood<sup>2, 6</sup> and tissues.<sup>7-10</sup>

The capacity of feces to produce nitrite from bismuth subnitrate was substantiated experimentally. Bohme<sup>8</sup> found that aqueous suspensions of the feces of children, and, less consistently and in a lesser degree, of adults after admixture of bismuth subnitrate, on incubation gave strongly positive nitrite reactions, and on injection into the alimentary canals of cats and rabbits resulted in the excretion of nitrite in the urine. Zadek<sup>11</sup> incubated the feces in diverse dietary modifications and pathologic conditions with one-tenth its weight of bismuth subnitrate, and detected nitrite in distilled water extracts of a fair proportion of adult specimens and in the greater number of specimens of children. The administration of bismuth subnitrate to a child with a gastrointestinal disorder on one occasion resulted in nitrite containing feces. With this exception the administration of large daily doses of bismuth subnitrate resulted in feces, which was nitrite negative on passage and which remained nitrite negative after

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incubation The capacity of such feces to produce nitrite on addition of bismuth subnitrate and incubation was unimpaired

Nitrite poisoning by enteric bismuth subnitrate was duplicated in animal experiments by Nowak and Gutig<sup>10</sup> who elicited the complete syndrome in cats

The material was reviewed by Beck,<sup>12</sup> Zollinger,<sup>13</sup> and Mayer and Baehr,<sup>14</sup> who accepted acute nitrite poisoning following the internal administration of bismuth subnitrate as proven

#### BISMUTH SUBNITRATE IN ARTERIAL HYPERTENSION

The nitrite effect of bismuth subnitrate by mouth, as exerted by small doses, has been applied therapeutically in arterial hypertension by Stieglitz<sup>15-18</sup> In essential hypertension, more especially when uncomplicated by advanced arteriosclerosis, the average systolic pressure in large series reverted far toward the maximum normal for the average age, and the diastolic approached normal In the hypertension of pregnancy the blood pressure elevation was moderated and its progress toward parturition arrested in the large majority of cases While the data were not controlled with regard to an adequate regulated period of pre-treatment observation, the effect of nonspecific medication, uniform frequency of visit during the treatment and pre-treatment periods, and a standardized procedure in determining the blood pressure, in essential hypertension,<sup>19 20</sup> or by parallel series without specific medication in the hypertension of pregnancy, these results are suggestive \*

The blood pressure reduction following the administration of bismuth subnitrate by mouth might be anticipated to be within certain limits dependent on the dosage The procedure was accordingly adopted of increasing the dosage by steps until the maximal effect was attained Such administration of graded doses gave indications of a clearcut relationship between dosage and decrease in blood pressure

#### CASE REPORT

*History*—H C C, male, aged fifty three, an engineer engaged in the transaction of clerical business, who had previously been free from major illnesses, and was in apparently good health, in the early afternoon of June 24, 1930, suddenly felt himself staggering During the course of the following morning an abrupt violent attack of nausea and vomiting occurred The nausea lasted throughout the day Dizziness on walking persisted Three days from the onset of symptoms the blood pressure was 200 systolic, 110 diastolic (Chart 1) The maximum concession made in restricting activity consisted in coming to the office in mid morning, and leaving by noon or mid afternoon The patient was regular in habits, ate sparingly, little meat, largely vegetables and starches, drank a quart of buttermilk a day, smoked heavily, and habitually took no exercise A solution of potassium iodide, gr viii, later grt x, t i d, was prescribed, and subsequently supplanted by ferric iodobenenate, gr viii, t i d, with such effect that the coriza of iodism by several weeks outlasted their administration Phenobarbital, gr ss, t i d, was taken over a long period without subjective notice Meanwhile the blood pressure mounted to 224 and subsided to 204 systolic, and mounted to 124 and found a level at 102 diastolic The dizziness had in a little over a month receded to infrequent slight spells, which decreased until they remained only in the traces of

\*As the result of the study of a small series in which these factors were taken into account a man<sup>19</sup> more recently concluded that bismuth subnitrate in such dosage exerts no demonstrable effect in essential hypertension although in the majority of cases the frequency of period was greater than in the entire period of previous observation and in the immediate foreground of a bismuth subnitrate effect.

some passing uncertainty on rising, which finally disappeared. Thenceforward the course was asymptomatic. Normal activity was resumed, except that an occasional freedom was accepted in coming to work late and leaving early, and extremes of over exertion were avoided. Cueurboectrin<sup>7</sup> 750 mg, t i d, was taken without altering the trend of the systolic pressure, but when the blood pressure readings were in addition made at weekly instead of bi weekly intervals, it fell 10 points,<sup>10</sup> only to rise 5 points when the blood pressure was not taken for a month. The diastolic pressure fluctuated about a mean of 104 during this period. All medication was discontinued. The patient, by direct inquiry and by various shifts, had secured the opinions of different physicians as to the eventualities and hazards of his malady, and retailed their diverse pronouncements with uniform humor and delight, seemed happy to be in a position to observe the course of his disease and content to accede to whatever might be its outcome, and felt considerable amateur interest in the various proposed methods of therapy, encouraged their trial, and was ready to cooperate faithfully in their prosecution.

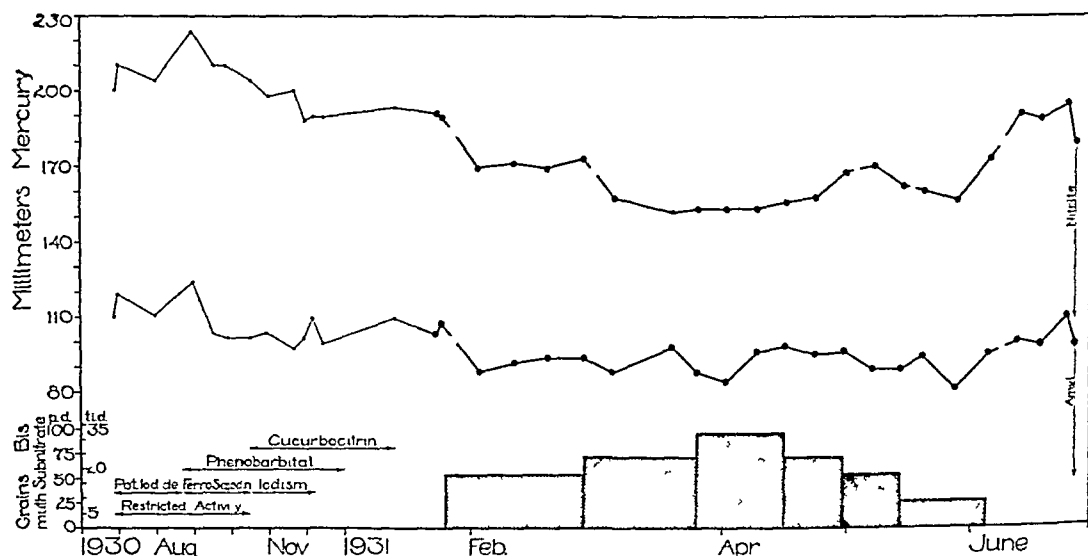


Chart 1—Graphic case report of arterial hypertension treated with bismuth subnitrate

**Physical Examination**—Middle aged man, average height and weight, approximating athletic habitus, fair sedentary musculature, some want of turgor in the skin and subcutaneous tissues of the face, slight pallor of the mucous membranes. Palpable arteries, compressible, nonresistant, not tortuous. Retinal arterioles, pale, moderately reduced caliber, slightly roughened outline, straight, branching at sharp angles, stiff and rigid looking, veins indented by the passage of the arterioles over them and bulging slightly. Apex beat palpable to the midclavicular line in the sixth interspace. Inconstantly a soft, but somewhat roughened, blowing nontransmitted systolic murmur at the apex.

**Laboratory Tests**—Urine, routine specimens, specific gravity, 1015-1034, consistently a trace albumin, infrequent hyaline casts. Blood chemistry: urea nitrogen 10.6, uric acid 3.5, glucose 92 mg per 100 cc. Blood Wassermann negative. Basal metabolic rate plus 4 per cent by the Boothby and Sandiford revision of the Du Bois standards.

**Therapeutics**—The blood pressure was determined after rest periods of some minutes to a half hour, the duration of which depended upon office exigencies. Successive readings were made, usually to the number of ten, during an interval of several minutes, or until they no longer tended downward but fluctuated on a level. The duplicate readings at this level were averaged. This conduced to securing comparable, partially controlled, semibasal pressures.

The blood pressure was determined weekly. Most readings were made on Wednesdays, but a few on Mondays and Fridays. All were taken in the mid or late afternoon.

The average range between the maximum and minimum readings at a sitting was 26 systolic, 8 diastolic<sup>21 22</sup> On one occasion after the patient had for the previous half hour been unable to escape the annoyance of an insistent salesman the range was 46 systolic, though but average range diastolic

The initial blood pressure determinations located a mean at 191 systolic, 106 diastolic

No change in the dietetic, hygienic, or psychic regimen was advised The continuance unmodified of the prevailing routine was encouraged

Bismuth subnitrate, in capsules or chartulae, t i d, apportioned approximately q 8 h to conduce to an even distribution through the twenty-four hours, was prescribed in graded dosages over a period of ten months

The initial dosage of gr xviii was continued for over one month During this interval the systolic pressure assumed a mean level of 172 The dosage was increased to gr xxiv for one month, further increased to gr xxxii for three weeks, and returned to gr xxiv for two weeks During these intervals the systolic maintained a mean of 157 The dosage was returned to gr xviii for two weeks During this interval the pressure reverted toward its earlier level under this dosage with readings of 171 and 163 The dosage was reduced to gr ix for three weeks On the first and second visits during this interval the rest period was prolonged, and relaxation was very complete but on the third it was of usual length The respective readings were 160, 157, and 173 Some partial remission in the hypertension<sup>23 24</sup> may have entered into this irregularity

The diastolic pressure assumed a mean level of 92 about which it fluctuated as long as bismuth subnitrate was continued

During the treated period the average lability range was 14 systolic, 7 diastolic After several hours of rush and strain, unmoderated by any intervening rest period, the range was on one occasion 36 systolic, 19 diastolic, even though the prevailing systolic level was not reached, but on another occasion, when neither the prevailing systolic nor diastolic was reached, no more than average Once after a few moments of heedlessly vigorous exercise a fall of 65 systolic, 24 diastolic, occurred in regaining the prior level over an interval of seven minutes

Bismuth subnitrate was discontinued for three weeks The blood pressure reverted to approximately its initial mean level with 188 systolic, 103 diastolic

During the combined untreated periods, the mean blood pressure was 189 systolic, 104 diastolic During the interval of maximal effective dosage of bismuth subnitrate the mean systolic was 157—a reduction of 32, or 16.9 per cent On decreasing the dosage the systolic pressure tended to revert to the level it has previously assumed with such dosage Throughout the period of bismuth subnitrate administration the mean diastolic was 92—a reduction of 12, or 11.5 per cent On discontinuing bismuth subnitrate the blood pressure resumed its initial level

When the blood pressure is plotted against the dosage of bismuth subnitrate and smoothed median curves drawn (Chart 2), the systolic pressure and its percentile reduction follows a skew sigmoid curve which runs into a horizontal line beyond gr xxi, the diastolic a hyperbolic curve which runs into a horizontal line beyond gr x

As a comparative test of the maximum blood pressure reduction resulting from acute extreme vasodilatation, the patient vigorously and exhaustively in-

haled 5 minims of amyl nitrite, with the result that the blood pressure at the approximate instant of maximal blushing and pulse acceleration<sup>3</sup> read 108 systolic, 43 diastolic. The maximal reduction obtainable with bismuth subnitrate was thus less than the maximum reduction effected by amyl nitrite. The limitation in the maximal effects of bismuth subnitrate was, therefore, not due to a non-dilatability of the hypertensive or sclerosed arterioles.

Freshly voided urine invariably tested negative for nitrite.

With the larger dosages some slight temporary intestinal retardation was experienced, which was readily counteracted by occasional small doses of magnesium oxide and later in some way compensated without it.

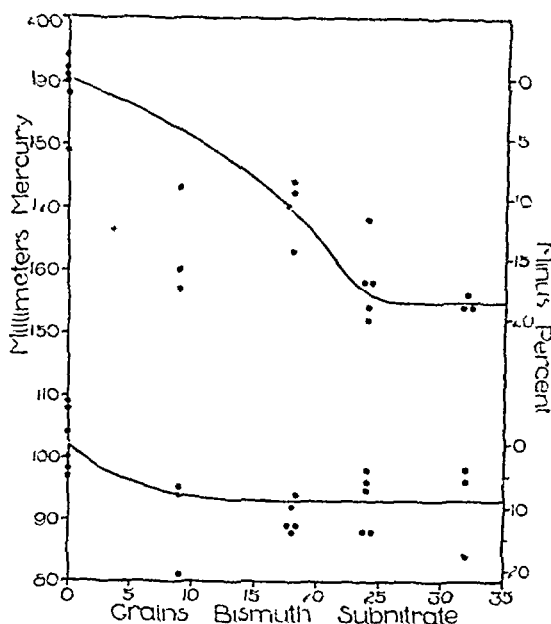


Chart 2—Relationship of dosage of bismuth subnitrate to blood pressure in case of arterial hypertension

Even when the fourth pound of bismuth subnitrate was being consumed the gums gave no evidence of a metallic line, although there was an early pyorrhea of the lower incisors to furnish the requisite sulphide.

The administration of the maximal effective dosage of bismuth subnitrate was resumed; the blood pressure resumed the level it had previously maintained under this medication.

**Diagnosis**—Essential hypertension moderate blood pressure elevation, hypertensive blood pressure lability,<sup>21, 24</sup> retinal arterioles of hypertension with arteriosclerosis,<sup>25, 26</sup> moderate left ventricular hypertrophy, slight renal involvement, asymptomatic.

**Summary**—Bismuth subnitrate by mouth was accompanied by a reduction of the systolic pressure which increased with dosage until the maximal was reached, a uniform reduction in the diastolic pressure, and a decrease in blood pressure lability.

Bismuth subnitrate by mouth thus appears to reduce the blood pressure in arterial hypertension and inhibit its lability. The greatest effect obtainable is

limited This limitation is not attributable to an inability of the arterioles to dilate The blood pressure reduction might be conditioned by the intermediary processes through which bismuth subnitrate exerts its nitrite effect The limitation might be secondary either to a limitation in the capacity for the conversion of bismuth subnitrate into nitrite in the alimentary canal, or to a limitation in the response of the hypertensive arterioles to nitrite produced in such quantity and at such a rate

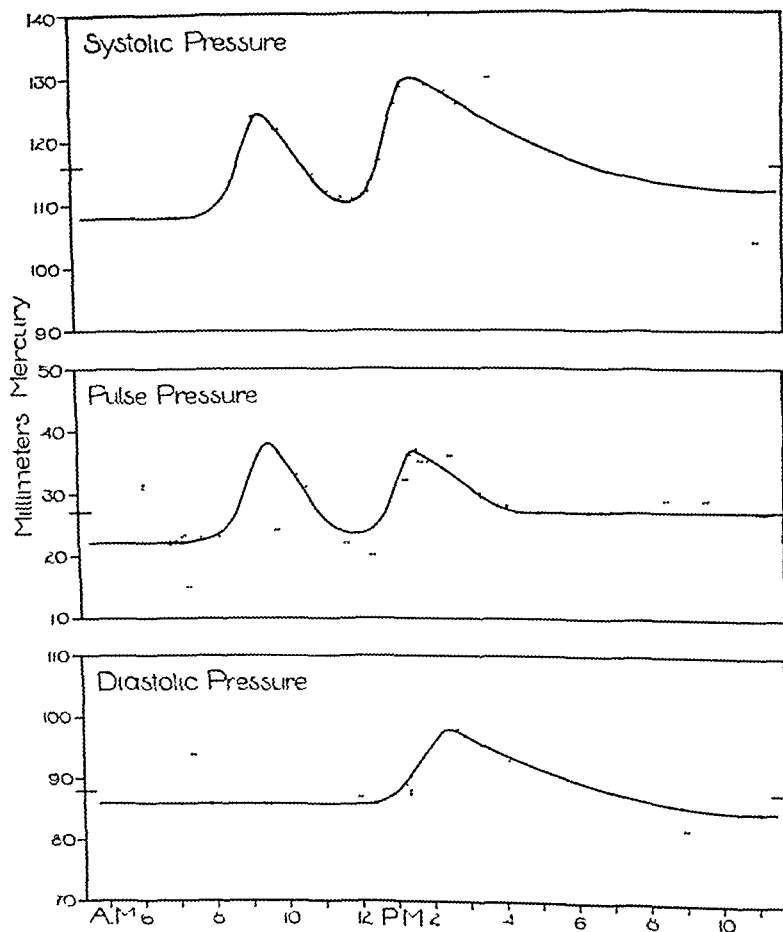


Chart 3—Diurnal blood pressure variation in normal arterial tension

Investigation of the temporal course and relative extent of the blood pressure lowering effect of graded dosages of bismuth subnitrate should demonstrate the potentialities and define the limitations inherent in this means of medication

#### EXPERIMENTAL

Since the nitrite effect of bismuth subnitrate by mouth might not supervene for some hours and should continue over an indefinite period, and inasmuch as the blood pressure traverses a diurnal cycle <sup>36 37</sup> it is not feasible to determine



the pressure, administer the drug subtract subsequent determinations from the original reading, and attribute the difference to the drug, assuming that without it the blood pressure would on the average have remained constant as with the sooner and more briefly acting members of the nitrite group<sup>35-40</sup> Since the

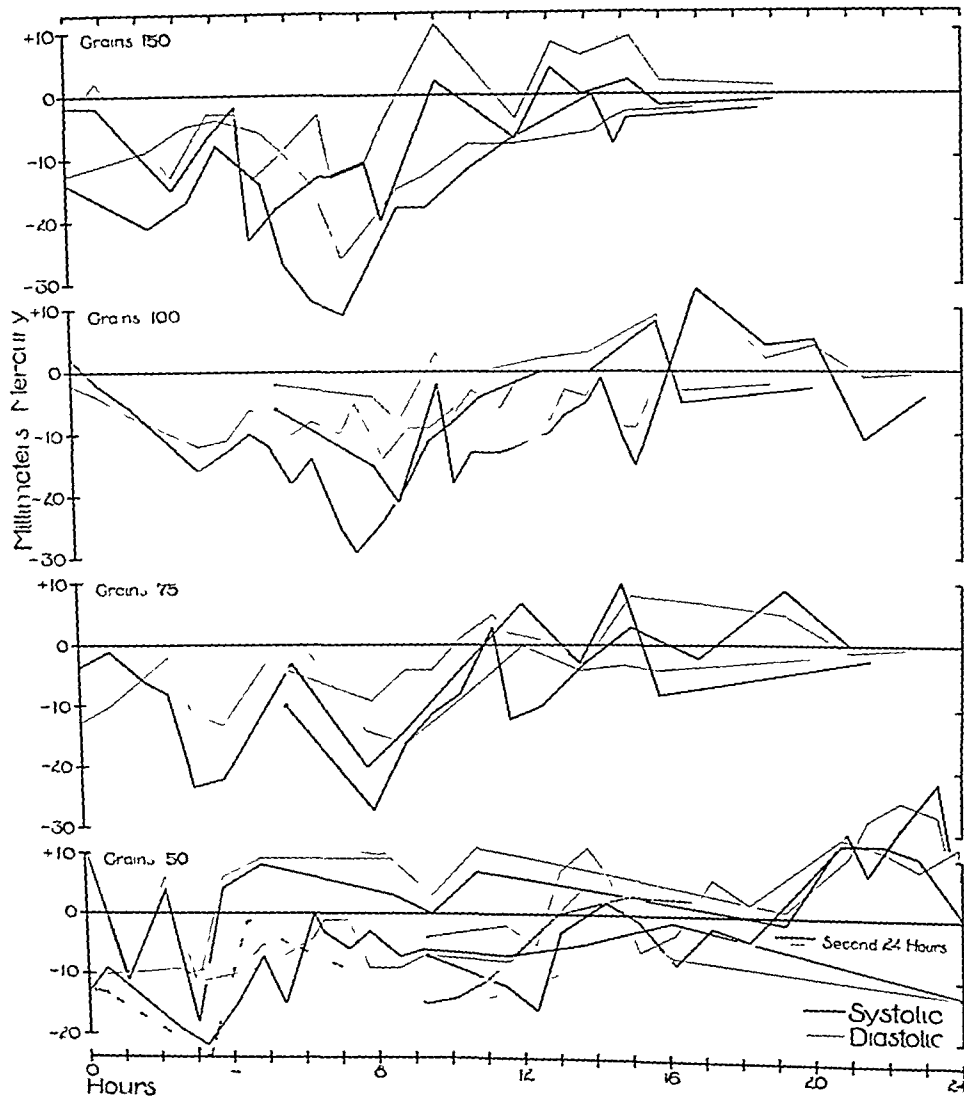


Chart 4—Deviation of blood pressure from median after bismuth subnitrate by mouth in normal arterial tension

blood pressure is less variable and erratic than in hypertension<sup>37</sup> more consistent data should be obtainable in normal arterial tension. Since it would be necessary to follow the blood pressure closely over a long period in order to establish adequate controls and conduct sufficient tests, it seemed more practicable to pursue the experiment in person.



On five consecutive and twenty one alternate days (Table I) the blood pressure was determined so far as practicable once during every waking hour. The pressures were taken with only such interruption of activity as was necessary to their taking. Each pressure was the average of five successive readings. While each reading was being noted down, the air was

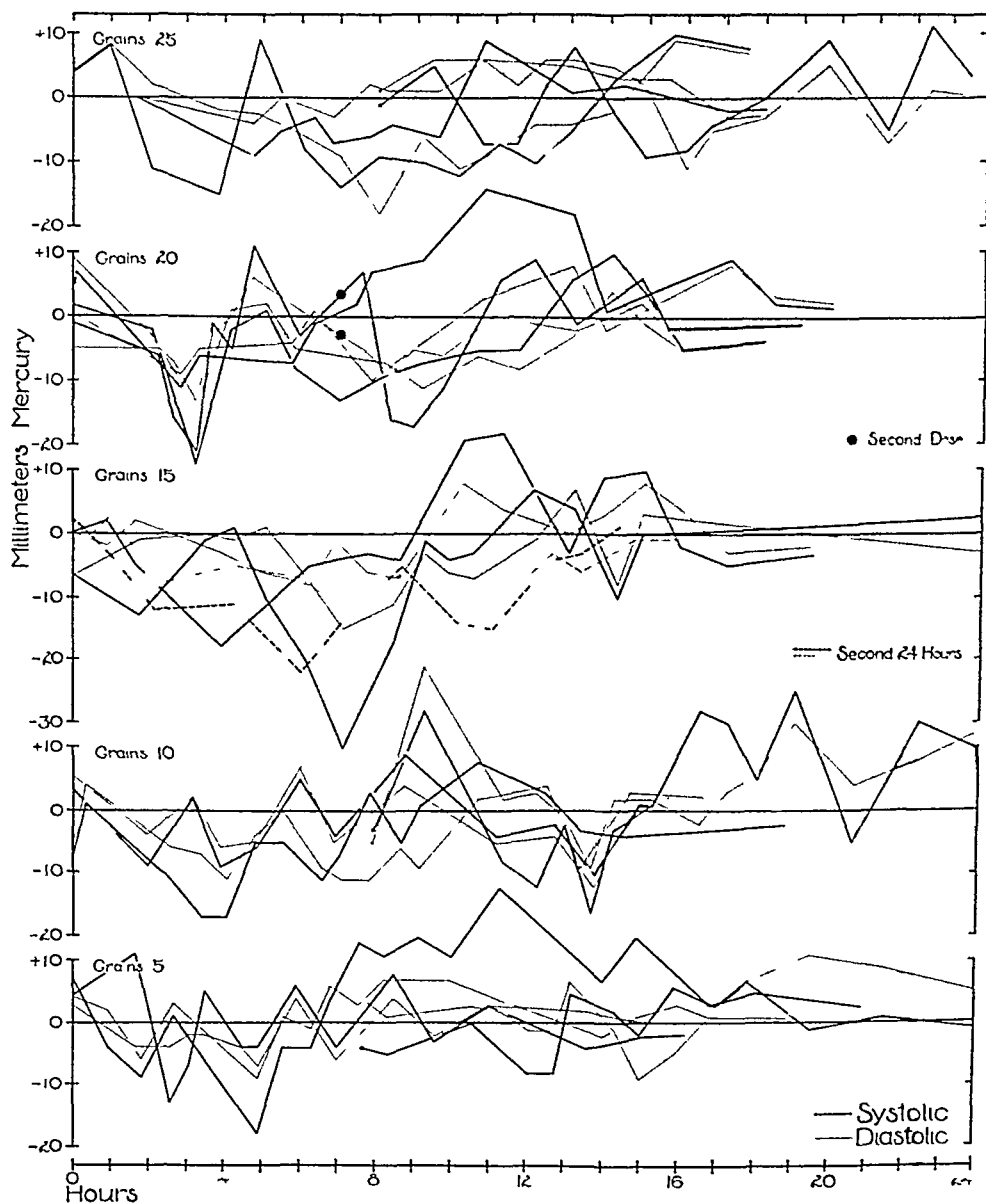


Chart 5—Deviation of blood pressure from median after bismuth subnitrate by mouth in normal arterial tension

allowed to escape freely from the cuff, so distal circulation might be reestablished. When indicated the procedure was modified to insure securing a representative value for the prevailing pressure. Occasionally a low initial reading resulted from the tendency to hold the breath and prolong inspiration while watching the pointer or the mercury column and waiting to

here the changes in the sounds. Infrequently some external disturbance or interruption caused a few high readings. Such aberrant readings were cancelled. Whenever the successive readings appeared to be unusually divergent additional readings were made. The several readings were averaged to the nearest millimeter, the time was recorded to the nearest twelfth hour. The diastolic, pulse, and systolic pressures were plotted against the hour of the day on scatter diagrams, and smoothed curves drawn through the median pressures of consecutive hours (Chart 3). The establishment of the trend and variation of the diastolic and systolic pressures through the day constituted a basis for comparison of the pressures following the administration of bismuth subnitrate.

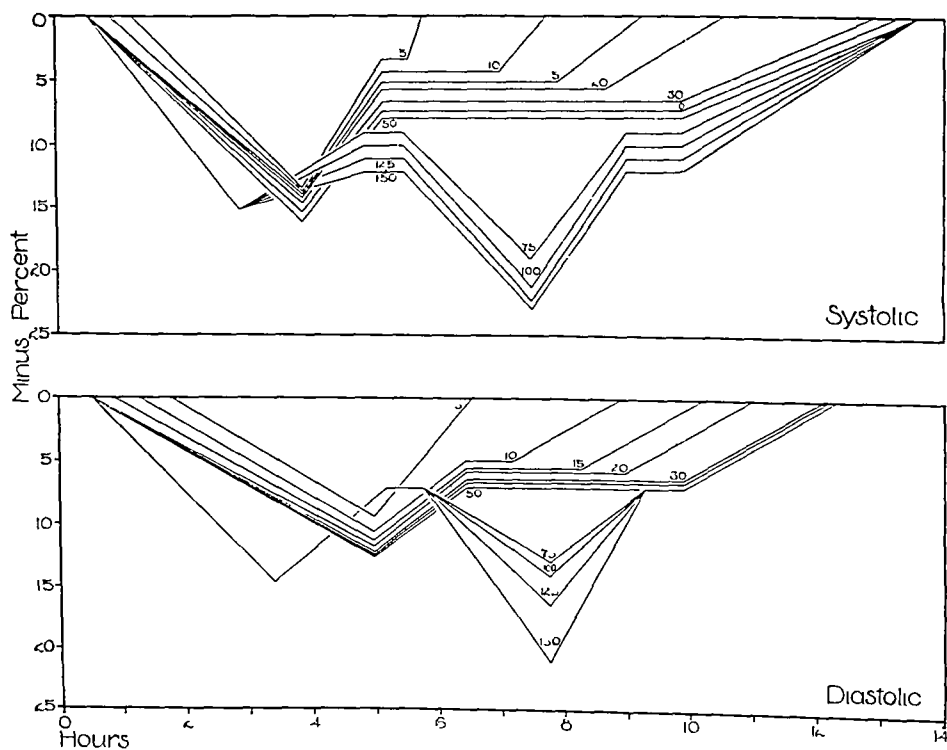


Chart 6—Blood pressure lowering effect of bismuth subnitrate by mouth in normal arterial tension

Graded doses of bismuth subnitrate, the supernatant fluid of distilled water suspensions of which tested negative for nitrite, were taken as powders, followed by a little water, and, in the bulkier amounts, by a few mouthfuls of food, to aid in swallowing, in random sequence, at the beginning of the intervening days or during the antecedent nights (Table I). The blood pressure was determined at frequent intervals. Each diastolic and systolic pressure was converted into millimeters plus or minus deviation from the median pressure for the same hour. The successive deviations with each dose were plotted against hours from administration and connected by straight lines (Charts 4, 5). From the original data and these charts, the period of submedian pressure, the median deviation in millimeters during this period and its percentile divergence from the median of the pressures of the trend on successive hours, the maximum deviation in millimeters and per cent, and the time of its occurrence, systolic and diastolic in each test were derived (Table I). These and additional statistics were generalized by plotting the times of beginning deviation, maximum deviation, discontinuance of median deviation, and cessation of deviation, and the maximum and median deviation in both millimeters

meters and per cent,<sup>20</sup> systolic and diastolic, against dosage, and calculating averages or drawing smoothed curves as indicated. Readings from these averages and trends were recorded (Table II) and incorporated in a schematic diagram (Chart 6).

Throughout the period of investigation all stools were described with respect to time of passage, gross appearance, presence of recognizable vegetable remnants, and occurrence of bismuth discoloration. The number of hours after the administration of a dose of bismuth subnitrate of the initial appearance and of the complete evacuation of the corresponding segment of bismuth greyed or blackened feces was recorded (Table I). Tests for nitrite were in some instances made on filtrates of aqueous suspensions of bismuth containing stools.

TABLE II  
BLOOD PRESSURE LOWERING EFFECT OF BISMUTH SUBNITRATE BY MOUTH  
NORMAL ARTERIAL TENSION

PRESSURE	DOSAGE	ONSET	PRIMARY MAXIMUM			SECOND MAXIMUM			LEVEL MAINTAINED			CESSATION
			hr	mm Hg	per cent	hr	mm Hg	per cent	mm Hg	per cent	hr	hr
Systolic	5 gr	1½	4	15.4	13.8				40	35	5½	5½
	10	½	4	15.7	14.0				51	45	7	7½
	15	½	4	16.0	14.2				60	53	7½	9½
	20	½	4	16.4	14.3				67	59	8½	10½
	25	½	4	16.8	14.4				73	64	9½	11½
	30	½	4	17.1	14.7				78	69	9½	12½
	40	½	4	18.1	15.3				86	75	9½	13½
	50	½	4	19.4	16.1				92	81	9½	13½
	75	½	3	19.0	15.2	7½	23.4	19.1	10.4	9.1	9½	13½
	100	½	3	19.0	15.2	7½	26.0	21.3	11.6	10.2	9½	13½
	125	½	3	19.0	15.2	7½	27.7	22.4	12.8	11.2	9½	13½
	150	½	3	19.0	15.2	7½	28.8	23.0	14.0	12.3	9½	13½
Diastolic	5	1½	5	8.0	9.4							6½
	10	1½	5	9.5	10.6				4.4	5.2	7	8½
	15	1	5	10.2	11.4				4.8	5.7	8½	10
	20	½	5	10.6	11.8				5.0	5.9	9	11
	25	½	5	10.9	12.1				5.3	6.2	9½	11½
	30	½	5	11.1	12.3				5.4	6.4	9½	12
	40	½	5	11.2	12.5				5.7	6.7	9½	12
	50	½	5	11.3	12.6				6.0	7.1	9½	12½
	75	½	3½	13.0	14.7	7½	11.8	13.0	6.0	7.1	9½	12½
	100	½	3½	13.0	14.7	7½	12.8	14.1	6.0	7.1	9½	12½
	125	½	3½	13.0	14.7	7½	14.9	16.4	6.0	7.1	9½	12½
	150	½	3½	13.0	14.7	7½	19.0	21.0	6.0	7.1	9½	12½

For alternate dosages of bismuth subnitrate urine specimens were collected from the preceding hour to the twenty-fourth hour after administration and saturated with an excess of chloroform to prevent bacterial decomposition. To test for nitrite 0.5 cc of sulphuric acid and of a naphthylamine acetate reagent<sup>22</sup> were added to 10 cc of each specimen. The reaction proceeded in the presence of chloroform. Under the conditions the test was sensitive to from 0.0001 to 0.0002 mg  $\text{NaNO}_2$  per cc. The nitrite content of urine did not alter appreciably during such intervals as elapsed between passage and examination. The specimens were measured and their twenty-four hour volumes totaled.

Supplementary tests were undertaken to estimate the sodium nitrite equivalents of the blood pressure lowering effects of bismuth subnitrate by mouth, and to secure comparative information on nitrite excretion with the two drugs. During the morning hours when the blood pressure maintains a level (Chart 3), its height was determined, graded dosages of sodium nitrite, tablets of which were freshly dissolved in small amounts of water, followed by the

rinsings of the glass, taken, and the blood pressure read every few minutes until its fall had passed a minimum. The systolic and diastolic reductions expressed as percentages of the original pressures<sup>35</sup> were plotted against dosage and smoothed curves drawn. Urine specimens were collected for the first half hours following the administration of sodium nitrite and for several subsequent hours (and in the first instances through twenty four hours). Their volumes were measured. To 10 cc of each specimen 0.5 cc of each reagent was added. The tint appearing in a positive test lighted through an equal depth of distilled water was matched with that of sodium nitrite standards viewed through a like volume of the urine specimen equally diluted. The nitrite content of the single specimens and of the several specimens following each dose were computed. The sodium nitrite excretion in mg and per cent, and the actual and graduated systolic and diastolic blood pressure reductions were recorded (Table III).

#### DIURNAL BLOOD PRESSURE CYCLE

The blood pressure traverses a diurnal cycle. In a group of college students in whom the blood pressure was followed during an evening and a day, Weyssse and Lutz<sup>36</sup> found a slight tendency toward a lowering of the diastolic pressure through the day, and a gradual increase in the systolic and pulse pressures, which, in addition, rose immediately after the ingestion of food and gradually declined till the beginning of the succeeding meal. In a group of ambulatory ward patients in whom the blood pressure was taken hourly during twenty-four hours, Mueller and Brown<sup>37</sup> found a gradual rise in the late afternoon and early evening, a gradual drop to a minimum at 4 A.M., and a return to the morning level in the systolic, similar but smaller changes in the diastolic, and rises after meals.

The median of 252 blood pressure determinations (Chart 3) during the waking hours of five consecutive and twenty-one alternate days over a period of seven weeks was 88 diastolic, 27 pulse pressure, and 116 systolic.

The median diastolic maintains a level of 86 from waking until after noon, rises sharply to a peak of 108 before midafternoon, and gradually declines to 85 toward midnight. The pulse pressure begins the day at 22, soon rises at first gradually and then more abruptly to a peak of 38 shortly past midmorning, recedes to 24 by noon, rises sharply to a peak of 37 in the early afternoon, and declines toward late afternoon to 27, which it maintains through the evening. The resulting systolic pressure starts at 108, rises to 125, recedes to 111, rises sharply to 130, and gradually declines to 112. The medians of the readings from these trends on successive hours through the day are 85 diastolic, 27 pulse pressure, and 114 systolic.

The course of the blood pressure is intimately associated with the routine of activity. The earlier rise in the pulse pressure and in the systolic pressure begins on rising and breakfasting, reaches its peak on arrival at the office, and then subsides. The later rise in the pulse pressure begins a little before lunch and reaches its peak subsequent to arrival at an afternoon clinic. The concomitant rise in the diastolic pressure begins slightly later and reaches its peak an hour later. The second peak of the systolic pressure is the resultant of these two rises. No after dinner increase is sufficiently salient to influence the trends, but in the early evening hours points preponderate slightly above them. This represents the course of the blood pressure associated with the usual routine of

activity. When the routine was drastically transgressed blood pressure readings most often could not be taken.

But over week-ends when professional activities were interrupted, the course of the blood pressure altered radically. The peaks were diminished or levelled. The diastolic level was 1 mm. lower through the morning, the afternoon peak one-third its week-day altitude, and the decline to the usual late evening pressure very gradual. The peaks and trough in the pulse pressure were obliterated and replaced by a slight upgrade from the morning to the evening level. The systolic pressure accordingly began near its usual level, made a slight rise during the morning, mounted to a broad flat peak of 5 mm. in the earlier afternoon and receded but slightly to its late evening height.

TABLE III  
SODIUM NITRITE IN NORMAL ARTERIAL TENSION

DOSAGE	SODIUM NITRITE EXCRETED		MAXIMUM BLOOD PRESSURE REDUCTION			
			SYSTOLIC		DIASTOLIC	
			ACTUAL	TREND	ACTUAL	TREND
gr	mg	per cent	per cent	per cent	per cent	per cent
0.5	0.0060	0.0185	3.8	5.4	0.0	0.0
1.0	0.0000	0.0000	9.8	7.7	0.0	0.0
1.5	0.0172	0.0176	15.2	9.0	6.4	0.2
2.0	0.0000	0.0000	7.9	10.0	0.0	0.7
2.5	0.0026	0.0016	11.4	10.8	2.7	2.8
	0.0296	0.0181	4.0		0.0	
3.0	0.0246	0.0126	19.1	11.3	18.2	7.2
	0.0717	0.0368	9.3		7.2	
3.5	0.0893	0.0393	13.3	11.9	11.4	8.4
	0.0000	0.0000	5.8		6.1	
4.0	0.0049	0.0019	16.0	12.3	10.4	8.7
	0.0822	0.0316	10.2		7.9	
4.5	0.0413	0.0141	11.3	12.6	6.4	8.8
	0.0736	0.0252	13.4		10.6	
5.0	0.0214	0.0066	8.8	12.8	9.9	8.9
	0.0886	0.0272	14.0		7.8	

During the week no distinctively high pressure days or low pressure days occurred. But days of stability in which the blood pressure followed its trends varied with days of instability in which it oscillated widely and erratically across the medians. Often the pulse pressure tended to adhere to its trend, even though the diastolic, and as a result the systolic, pressure fluctuated.

The scatter of the individual points is wide. It is greatest for the systolic, less for the diastolic, and least for the pulse pressure. But almost all points (respectively 83, 88, and 91 per cent) lie within plus or minus 10 mm. The large majority (57, 59, and 67 per cent) lie within plus or minus 5 mm. No increase in the scatter accompanies increase in pressure.<sup>43</sup>

The blood pressure is very labile even with normal arterial tension.<sup>44</sup> Considerable minute to minute fluctuation appeared in the successive readings in a determination. The range between maximum and minimum readings was from

1 to 24 mm for the systolic and from 2 to 23 mm for the diastolic. The mean was 9.6 systolic, and 9.7 diastolic. The modes were both 8, the medians 9. The lower quartile was 6 and the upper 12 systolic, and 7 and 12 diastolic. These distributions are almost identical for systolic and diastolic pressures.

#### BISMUTH SUBNITRATE IN NORMAL ARTERIAL TENSION

Bismuth subnitrate by mouth lowers the blood pressure in normal arterial tension (Charts 4, 5, Table I). The rapidity, intensity, and duration of the effect increase with dosage (Table II, Chart 6).

The effect begins as late as during the second hour with the smaller dosages but in one-half hour with moderate and larger dosages.

With small and moderately large dosages, the maximum effect on the systolic pressure occurs at four hours, on the diastolic at five hours.

But with the bulkier dosages an earlier maximum occurs at three hours in the systolic, at three and one-half hours in the diastolic, a partial recession intervenes, and a later maximum follows at seven and one-half hours systolic, seven and three-fourth hours diastolic.

Beyond the principal maximum the effect decreases to a level it maintains until from the sixth hour with the smallest dosage to nine and three-fourth hours with moderate and larger dosages.

The effect ceases after from five and three-fourth hours for the smallest dosage to thirteen and one-half hours for the larger dosages in the systolic, and from six and one-half to twelve and one-fourth hours in the diastolic.

The principal maximum in the systolic increases from 15.4 mm or 13.8 per cent with the smallest dosage, at a gradually increasing rate up to a large dosage, and beyond it at a gradually decreasing rate, approaching a limit of 29 mm or 23 per cent with the bulkier dosages. In the diastolic it increases, from 8 mm or 9.4 per cent, at a rapidly decreasing rate up to a moderately large dosage, and beyond it at a gradually increasing rate.

The primary maximum with the bulkier dosages is uniformly 19 mm or 15.2 per cent in the systolic, 13 mm or 14.7 per cent in the diastolic. These values are similar to those of the next lower dosage giving a single maximum. The second maximum increases in continuity with the primary maximum of lesser dosages.

The sustained effect increases in the systolic, from 4 mm or 3.5 per cent with the smallest dosage, at a gradually decreasing rate up to a large dosage, and then at a uniform rate, in the diastolic it increases, from 4.4 mm or 5.2 per cent with the smallest dosage giving it, over the same interval in a lesser degree, and then remains constant at 6 mm or 7.1 per cent.

Thus the increase of the blood pressure lowering effect of bismuth subnitrate by mouth with dosage encounters several limits in normal arterial tension. The rapidity of onset reaches a temporal limit with small or very moderate dosage, the continuance of the sustained effect with moderate dosage, and the duration with moderately large dosage. Although their times are fixed, the dosage determines the occurrence of one or two maxima. The sustained diastolic effect

reaches an intensive limit with moderately large dosages, and the systolic maximum beyond the bulkiest dosage

The lability range of successive readings during the intervals of blood pressure reduction was from 2 to 30 for the systolic, from 3 to 22 for the diastolic. The mean was 10.5 systolic, 9.5 diastolic. The modes were 8. The median was 10, the lower quartile 7, the upper 14, systolic, and 8, 7, and 12, diastolic. The systolic range was increased, the centering constants raised, and the interquartile interval extended. The diastolic range was slightly decreased but the centering constants were not significantly lowered.

#### CHEMISTRY AND PHARMACOLOGY

The basic bismuth nitrates arise from the hydrolysis of normal bismuth nitrate. The primary hydrolytic product is the bismuth oxynitrate monohydrate<sup>45</sup> which dissociates into hydrated bismuth oxide cations and nitrate ions, and into anions of the complex acid of the hydrate of undissociated bismuth oxynitrate and hydrogen ions. By combination and rearrangement these ions form a series of interconvertible intermediates and stable molecular and ionic aggregates of bismuth oxynitrate, bismuth hydroxide, water and nitrate, the constitution of which depends on the temperature and concentration of the solution. Bismuth subnitrate is a mixture of these basic bismuth nitrates.

Bismuth subnitrate reacts incompletely with hydrochloric acid in concentrations corresponding to normal or increased gastric acidity, at body temperature, to form the oxychloride and the soluble chloride.<sup>46</sup> After the ingestion of its basic salts, bismuth is accordingly absorbed in insignificant amounts, proportional to the degree of gastric acidity and then sojourn in the stomach, but irrespective of their duration in the intestines, and excreted by the kidneys,<sup>47</sup> salivary glands, cecum, and colon.<sup>48</sup>

Bismuth subnitrate is difficultly soluble in water. The nitrate in the basic bismuth salts has a different reaction capacity according to whether it is directly attached to bismuth or is attached to the aggregate as an ion which can be neutralized by dilute hydroxide.<sup>49</sup> Bismuth subnitrate in suspension in distilled water at body temperature furthermore sets nitric acid free immediately and in slowly increasing quantity by hydrolysis.<sup>46</sup>

After the ingestion of bismuth subnitrate nitrate is therefore available in appreciable concentration in the gastrointestinal tract.

The promptness of the onset of the blood pressure lowering effect suggests the possibility that nitrate absorbed under these conditions may be reduced in the tissues in sufficient quantity to produce an appreciable nitrite effect.

\*Free bismuth ions occur in suspensions of bismuth subnitrate<sup>46</sup> and are therefore also available for absorption by the intestine.

Yet bismuth subnitrate has been administered over long periods of time in arterial hypertension without giving rise to any symptoms or signs of intoxication.<sup>32-35</sup>

Despite its long established widespread and intensive use in gastrointestinal disorders only two clinical instances of metallic poisoning have been reported.<sup>17, 33</sup>

Chronic perhaps subclinical poisoning however may occur. Kidney lesions consisting of calcification of necrosing tubular epithelium<sup>44</sup> probably of long standing and produced by long-continued self-medication with bismuth compounds for gastrointestinal disease are an occasional incidental autopsy finding (personal communication).

Bismuth can be absorbed from the intestinal tract either through the production of its chloride or through ionization. If the production of the chloride is the major factor this could be obviated by administering bismuth subnitrate in enteric capsules so as to minimize if necessary its toxic effects.

Stepanow<sup>48</sup> found nitrite in certain tissues of dogs and rabbits on ordinary diet but not after a nitrate-free diet. After nitrate-free diet these tissues contained nitrite upon intravenous injection of sodium nitrate. Grinding nitrite-negative tissue with nitrate yielded nitrite. Bernheim and Dixon<sup>49</sup> showed that liver substance, and the muscle substance of some species, reduces nitrate to nitrite.

The unabsorbed nitrate is subject to bacterial action. In a substrate of alkaline reaction and in the presence of a sufficient abundance of organic nutrient material, numerous bacteria, including *B. coli communis*, *B. coli communior*, *B. acidilactici*, *B. lactis aerogenes*, and *B. alkaligenes faecalis*, actively convert potassium and other soluble nitrates into nitrites.<sup>50</sup> The completeness of the conversion and the rapidity with which it reaches its maximum increase with the dilution of the nitrate.<sup>51</sup> The reduction proceeds with the access as well as in the absence of oxygen. The presence of nitrate is without prejudice to the growth of the bacteria, and only on increasing its concentration is their development inhibited.<sup>50</sup>

Nitrate is reduced to nitrite in the alimentary canal. After massive poisoning with sodium nitrate Barth<sup>52</sup> found nitrite in the intestinal content of cattle. After instillation of nitrate isolated intestinal loops of dogs contained nitrite. After excessive dosages of sodium nitrate Binz and Gerlinger<sup>53</sup> demonstrated large amounts of nitrite in the small intestinal content of dogs and rabbits.

Bismuth subnitrate reacts like the soluble nitrates. In the presence of bismuth subnitrate cultures of *B. coli* produce nitrite.<sup>8, 10, 11</sup> The intestinal content of cats showing signs of nitrite poisoning after ingestion of bismuth subnitrate contains nitrite.<sup>10</sup>

Bismuth subnitrate maintains nitrate constantly present in the intestinal content in great dilution. In man the conditions favorable to the reduction of nitrate to nitrite obtain, and the requisite bacteria preponderate in the lower ileum and the colon. As the bismuth subnitrate reaches these parts, its free nitrate should accordingly be reduced, and the resulting nitrite absorbed. Nitrite does in fact arise from bismuth subnitrate in the large intestine.<sup>2, 5-11</sup> Nitrite is found in intestinal content containing bismuth subnitrate.<sup>9, 11</sup> Such nitrite is absorbed into the body fluids.<sup>8, 9</sup> Feces, with fewer viable, and more attenuated, bacteria than intestinal content, can still reduce bismuth subnitrate to nitrite.<sup>8, 11</sup>

The time relationships of the blood pressure lowering effect of bismuth subnitrate by mouth are affected by gastrointestinal motility. On the occasion when the bismuth subnitrate was taken the preceding evening and the effect lasted into the following day, and on the two occasions when it was taken in the early morning hours (Table I), the time of each phase of the blood pressure reduction, observed after an interval of several hours' sleep, was markedly delayed in comparison with when the drug was taken on rising and had the advantage of daytime peristalsis and a small aftercoming breakfast and luncheon (Chart 4).

The bismuth subnitrate and its products themselves influence intestinal motility. The times of both the initial appearance and the complete evacuation of bismuth feces increase with the dosage of bismuth subnitrate (Table I).



With the bulkier dosages of bismuth subnitrate a uniform primary maximum blood pressure reduction is, after an intervening recession, followed by a variable second maximum (Charts 4, 6, Table II). A certain mass of bismuth subnitrate, on reaching the site of nitrate reduction, may liberate such a quantity of nitrite, that peristalsis is retarded and further supply delayed. As this subnitrate expends its nitrite the inhibition is relaxed. When peristalsis resumes the remaining bismuth subnitrate is brought up to contribute its nitrite.

With lesser dosages of bismuth subnitrate the maximum blood pressure reduction is followed by a recession to a relatively sustained level (Charts 4, 6, Table II). The first arrival of bismuth subnitrate at the site of nitrate reduction may then liberate such a quantity of nitrite, that its continued advance is delayed, until an equilibrium is established between bismuth subnitrate supply and nitrite production.

The therapeutic benefits of bismuth subnitrate in diarrhea may be due less to its mechanical distribution over the mucosa than to its liberation of nitrite which depresses the muscularis.

Only during a small fraction of the time bismuth is present in the intestinal tract is the nitrite effect of its subnitrate exerted. The discontinuance of the effect is not due to exhaustion of the nitrite, for, if after the first blood pressure reduction, it happens that the equilibria among the intestinal flora are so deranged, that then biochemistry takes a turn toward putrefaction, a subsequent reduction ensues, at least equal to the original in duration and intensity (Table I, Charts 4, 5). The subsidence and cessation of the nitrite effect while nitrate is still available might be due either to the transport of the bismuth subnitrate to a part of the intestine unfavorable for its reduction to nitrite or to the further reduction of nitrite. In the presence of carbohydrate or the higher alcohols, the bacilli, which reduce nitrate to nitrite, carry its reduction beyond nitrite, themselves elaborating ammonia, while others liberate free nitrogen<sup>50</sup>. The entire fund of nitrate derived from bismuth subnitrate is finally either absorbed, or reduced to nitrite, which is either absorbed, or further reduced. Bismuth feces contains neither nitrite (Table I) nor nitrate to be reduced to nitrite.<sup>21</sup>

Bismuth subnitrate by mouth in large dosage, Mehitens, Hanzlik, et al.<sup>54</sup> observed, occasionally produces with controlled fluid intake, a diuresis, which is not due to the absorption of bismuth in view of the nonexcretion of bismuth in the urine, but is due to nitrite action as indicated by coincident nitrite effects on the circulation. With free fluid intake the dosage of bismuth subnitrate does not perceptibly influence the urinary output.

Bismuth subnitrate by mouth causes a reduction of the blood chlorides due to the chloride diuresis of nitrate.<sup>16</sup>

Nitrite is not excreted in the urine after bismuth subnitrate by mouth (Table I).

But sodium nitrite is partly excreted as nitrite (Table III). The excretion is irregular and minute. Even with dosages well in excess of the therapeutic, the total excretion, which tends to increase with dosage, did not exceed 0.089 mg or 0.039 per cent. The sodium nitrite content of the urine ranged from liminal

to 0.0035 mg per cc. Nitrite excretion was independent of urinary output, the diminution of which generally corresponded to the blood pressure reduction, for the smaller volumes of urine contained higher concentrations of nitrite. Although the initial excretion of nitrite often coincided with the peak of the blood pressure reduction, increased rapidity of blood pressure reduction did not conduce to increased nitrite excretion, and with a given dosage the amount of nitrite excreted was not related to the extent of the blood pressure reduction. Thus nitrite excretion did not seem to depend on the rate of its absorption, nor on renal factors, but on its metabolism. "The nitrate absorbed is excreted in the urine as nitrate although some of it may remain unoxidized."<sup>55</sup>

The overwhelming destruction and minute excretion of nitrite in the urine during and after the acute influx of nitrite into the blood stream incident to the absorption of sodium nitrite precludes the possibility of its excretion during the gradual prolonged access of nitrite derived from enteric bismuth subnitrate in limited dosages.

The blood pressure lowering effect of sodium nitrite in normal arterial tension (Table III) was irregular but in the systolic the increase in the maximum tended to become continuously less with successive increases of the dosage, as in an exponential curve, approaching a limit of 13 per cent, and in the diastolic to become greater and then less, as in a sigmoid curve, approaching a limit of 9 per cent.

Bismuth subnitrate if completely converted into nitrite would be equivalent to less than 24.1 per cent its weight of sodium nitrite. No inference as to the actual degree of its conversion is possible because the nitrite effect depends on the relative rates of absorption and destruction. But moderate dosages of bismuth subnitrate by mouth maintain a blood pressure reduction comparable with the maximum reduction of therapeutic dosages of sodium nitrite.

Nitrite absorbed as such does not produce tolerance.<sup>56</sup>

#### DISCUSSION

Arterial hypertension is mediated by increased arteriolar resistance.<sup>57-58</sup> Cardiac work is thereby increased.<sup>58</sup> As failure impends cardiac output and the velocity of blood flow decrease.<sup>58-60</sup>

The abnormal arteriolar resistance is largely functional and can be reduced by dilatation.<sup>61</sup> Sustained reduction of the arteriolar resistance should obviate the increased demand upon the heart, defer its late secondary effects on the circulation, and avoid the concomitant hypertrophy of the left ventricle<sup>61</sup> and the hastened development of arteriosclerosis.<sup>62</sup>

Among "the pharmacologic and therapeutic characteristics essential to a substance that is to be beneficial in arterial hypertension" is that it "must decrease the arteriolar resistance without disturbing essentially the other fundamental normal characteristics of the circulation, such as the cardiac output, the velocity of the blood flow, or the circulating blood volume. The effect of the substance and the persistence of its action on the blood pressure must be such that by repeated administration of the drug marked fluctuations in the blood

pressure are not produced. Establishment of a permanent lower level should occur gradually. The normal vasomotor responses essential to normal bodily functions should not be affected.<sup>63</sup>

Bismuth subnitrate by mouth effects a reduction of blood pressure. In intensity its effect is comparable to that of the other members of the nitrite group.<sup>39</sup> But the nitrite effect of the insoluble basic bismuth nitrates is more prolonged than even that of the higher alkyl nitrates.<sup>75-80</sup> The dosage can be manipulated to give the desired degree of blood pressure reduction. By repetition of the dosage at proper intervals a temporal summation of effect as in incomplete tetanus may be obtained. The blood pressure might by this means be maintained at a relatively constant reduced level. The nitrite action is exerted almost exclusively on the arterioles. Vasomotor responses, while moderated, remain intact.

### CONCLUSIONS

1 Bismuth subnitrate by mouth produces prolonged and effective reduction of the blood pressure in normal arterial tension.

2 Bismuth subnitrate by mouth is available for the therapy of arterial hypertension.

3 Bismuth subnitrate by mouth merits adequately controlled application in arterial hypertension to definitely evaluate its therapeutic efficacy.

### ADDENDUM

#### *Blood Pressure Lowering Effect of Inorganic Nitrates by Mouth*

Since the blood pressure lowering effect of bismuth subnitrate by mouth was investigated because of toxicologic<sup>1-14</sup> and therapeutic<sup>15-18</sup> rather than analytic considerations, the investigation was extended to other inorganic nitrates to determine the possible occurrence of similar effects.

Nitrate-reducing bacteria convert the nitrates of sodium, potassium, ammonium, and calcium to nitrite.<sup>50</sup> Sodium nitrate is reduced to nitrite in the animal intestine,<sup>52-53</sup> within the organism,<sup>48</sup> and by certain tissues.<sup>48, 49</sup> Daily diuretic doses of ammonium nitrate occasionally produce cyanosis, methemoglobinemia, and nitrite symptoms in man.<sup>64-67</sup>

The procedure developed with the basic bismuth nitrates for determining the blood pressure lowering effect of drugs acting during an indefinite period was reapplied with the normal nitrates of such metals as are relatively nontoxic by mouth.

The control blood pressures taken on alternate days were separately plotted and smoothed for week days and for week-ends. The week day diastolic and systolic pressures from about 7 A M to after 1 P M traversed the same cycles as previously (Chart 3), but in the diastolic the forenoon pressure was 2 mm lower and the afternoon peak 1 mm higher, and in the systolic the pressure was from 3 to 6 mm higher throughout. The week-end diastolic pressure maintained its previous morning level, but declined moderately during the afternoon, while the systolic began 2 mm higher, gradually rose 4 mm between 10 A M and 1 P M, and as gradually returned to its original level during the later afternoon.

The ratios of the weight of the nitrate radicals to the total molecular weight of the selected nitrates (Table IV) and their reciprocals were calculated. The reciprocals multiplied by 1, 2, and 5 gm fixed the amounts of comparable dosages of the several nitrates containing these equivalents of nitrate. Such dosages of nitrate-free chemically pure crystalline substances were weighed out to the nearest decigram (Table IV), freshly dissolved in small volumes of water, and gulped down, followed by repeated rinsings of the glass, to allay the taste in the mouth, and to avoid possible gastric irritation (which was manifested only with cerous nitrate which produced prolonged activation of the vomiting reflex), and after a short interval by a small breakfast.

The blood pressure responses were charted (Chart 7) and tabulated (Table IV).

The normal inorganic nitrates by mouth lower the blood pressure in normal arterial tension (Chart 7, Table IV). The effect is more intense and more consistent in the systolic than in the diastolic.

Nitrate equivalents of any of the alkalis, lithium, sodium, potassium, and ammonium, exert approximately equal effects. The nitrate equivalents of 2 gm have only a slightly more intense and more prolonged effect than the nitrate equivalents of 1 gm. The nitrate equivalent of 5 gm, however, exerts a very much more acute and more intense effect.

Nitrate equivalents of the alkaline earths, calcium and strontium, also exert comparable effects. With the alkaline earths the effect is more sustained, though little more intense, with nitrate equivalents of 1 gm, than with the alkalis. But the effect becomes much more acute and twice as intense on doubling the nitrate equivalent.

With magnesium the nitrate equivalent of 1 gm exerts an effect three times as intense as with the alkalis, or the alkaline earths.

With aluminum, which is 2.4 per cent hydrolyzed at 40° C<sup>68</sup> this nitrate equivalent gives a more prolonged and somewhat more intense effect than with the alkalis or alkaline earths.

But with ferric nitrate, which is also hydrolyzed, the effect is slightly less than with the alkalis.

The relative intensity and duration of the blood pressure lowering effects of equivalents of different nitrates and of different equivalents of the same nitrate by mouth might be conditioned by the solubility of the nitrate, the permeability of the intestinal mucosa to its cation, and its influence on intestinal motility.

The normal metallic nitrates are all fairly soluble. Their relative solubility is indicated by the number of gm anhydride per 100 gm aqueous solution<sup>68</sup> interpolated for body temperature (Table IV). Such differences in solubility as exist between the nitrates tested do not show any distinct influence on the blood pressure lowering effect. Barium, dissolving only 11.8 gm per 100 gm solution,<sup>68</sup> is relatively much less soluble than the other nitrates. But since the dosage required to secure a comparable effect would be twice the equivalent of the stated probable lethal dose of its chloride,<sup>69</sup> it might prove inexpedient to ingest it during the course of an experiment.

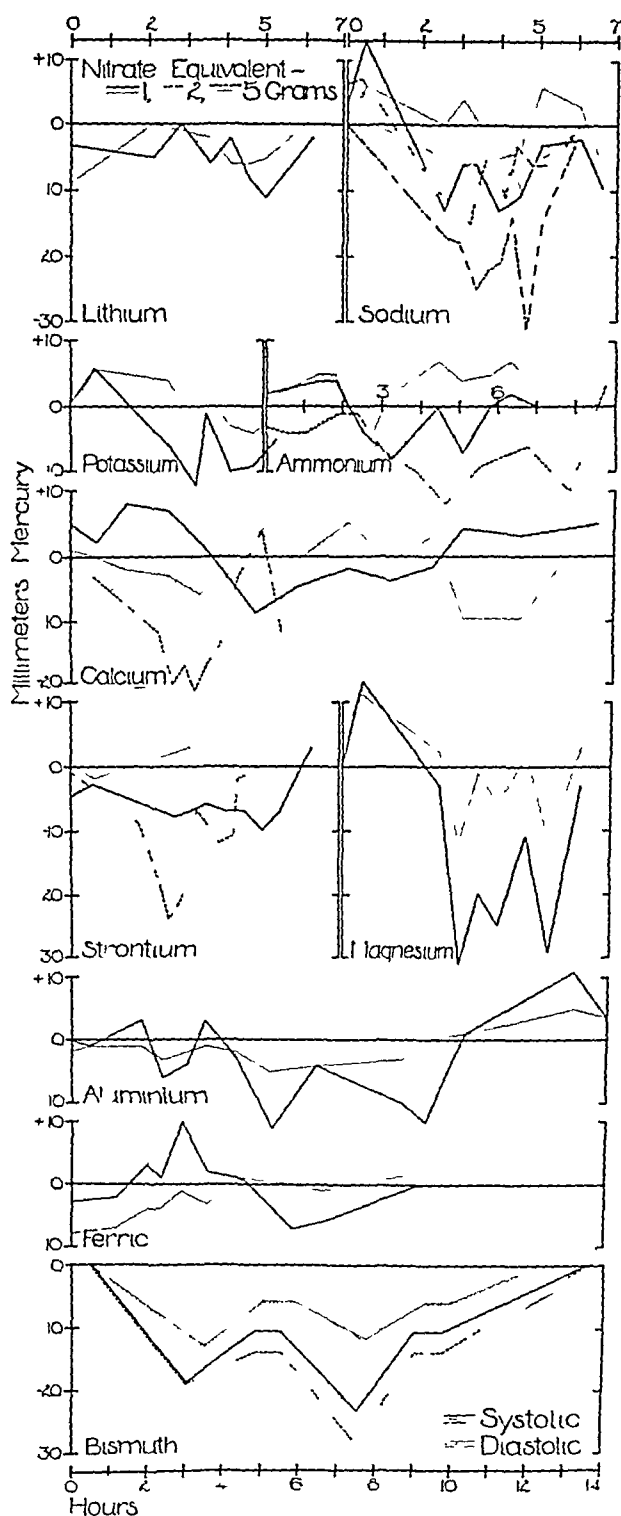


Chart 7—Deviation of blood pressure from median after administration of inorganic nitrates by mouth in normal arterial tension

TABLE IV

BLOOD PREPARATION FOLLOWING TREATMENT OF INORGANIC NITRATES BY MOUTRIE NORMAL ARTIFICIAL DILUTION

FORMULA	MOLECULAR WEIGHT	NITRATE RATIO $\frac{\text{NaNO}_3}{\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}}$	SOLUBILITY 500 mg. in 100 ml. solution	CATION DIFFERENCE	DOSE	STATISTICAL						DISTRIBUTION					
						MEDIAN			MAXIMUM			MEDIAN			MAXIMUM		
						ht	mm Hg	per cent	ht	mm Hg	per cent	ht	mm Hg	per cent	ht	mm Hg	per cent
Potassium	68.9	0.900	58.8	+++	1.1	1 - 6 1/2	7	5.9	5	11	9.3	2 1/12 - 6	5	6.0	1 1/1	6	7.2
Sodium	85.0	0.729	50.6	+++	1.1	1 1/2 - 6	6	5.2	2 1/2	11	11.0	1 1/2 - 2 2/3	1 1/2	5.6	1 1/12	5	6.0
					2.7	1 1/4 - 6	6	5.2	1 1/6	15	12.2	2 1/12 - 6	1 1/2	1.9	1 1/1	6	7.2
					6.9	0 - 6	18	15.7	1 7/12	11	21.6	1/1 - 6	6	7.1	1	12	11.1
Calcium	101.1	0.613	36.4	+++	1.6	1 7/12 - 6	7 1/2	6.1	1 1/6	12	9.6	2 11/12 - 5 5/6	1	1.6	2 2/3	1	1.8
Ammonium	80.0	0.775	73.6	+++	1.1	2 1/2 - 5 1/4	5 1/2	1.9	1 1/6	8	7.0		-5	-6.0			
					2.6	2 1/3 - 8 1/2	9 1/2	5.5	1 7/12	15	11.2	1 5/6 - 6 1/6	5	6.0	2 2/3	6	7.2
Strontium	230.2	0.525	63.8	±	1.9	1 7/12 - 9 7/12	1	1.6	1 3/4	9	7.9	2 1/3 - 1 1/6	3	1.6	1 1/1	6	7.1
					1.8	0 - 1 7/12	17	11.1	1 1/6	21	17.2	2 1/1 - 1 1/2	5	6.0	3 1/2	8	9.5
					1.7	7/12 - 6	7	5.9	1 1/12	10	8.6		0	0.0			
					1.1	0 - 5	11	9.1	2 1/2	21	19.0		0	0.0			
Barium	256.1	0.182	15.2	±	2.1	2 1/4 - 6 1/3	22 1/2	19.1	1 1/12	11	21.6	2 2/3 - 6 1/12	6	7.1	1 1/12	12	11.1
Lithium	58.9	0.632	16.0	±	1.6	2 - 10 1/4	6	15.5	1 1/4	14	12.1	1 5/6 - 9 1/4	1	1.6	5 1/4	5	6.1
Cesium	132.9	0.125	soluble	±	2.12												
Thallium	101.0	0.161	50.1	±	2.2	1 2/3 - 9 1/12	5 1/2	1.9	5 5/6	7	6.1		1	1.9			
Vanadium	101.0	0.201	insoluble	±	5.02	1/2 - 13 1/2	10.1	9.1	7 1/2	19.0	15.2	1 1/2 - 12 1/4	6.0	7.1	1 1/2	11.0	11.7
					9.9	1/2 - 11 1/2	11.0	12.1	7 1/2	28.8	16.2	1 1/2 - 12 1/4	6.0	7.1	7 1/4	11.8	11.0

1 Landolt-Bornstein physikalisch-chemische Tabellen

2 Hypermetris experimentalis

3 Table II, grades 75 and 150

The permeability of the intestinal mucosa for the cation might influence the blood pressure lowering effect by affecting the duration of the ionic nitrate within the intestine. While the alkalis, especially ammonium, are all highly permeable,<sup>67</sup> the alkaline earths, and magnesium, aluminium,<sup>70</sup> and iron while penetrating the intestinal wall in minute amounts, are almost totally excluded<sup>55-60</sup> (Table IV). The less permeable cations conduce to more prolonged and more intense effect, and to greater increase of the effect with increase in dosage. This tendency is offset by hydrolysis.

The ability to excite peristaltic waves manifested by magnesium and the higher dosages of the other salts, is a more decisive factor. While impermeability of the cation may tend to detain the nitrate ion in the intestine, stimulation of peristalsis propels it directly to the site for nitrate reduction. Thus moderate catharsis increases the nitrite effect.

The nitrite effect of the normal inorganic nitrates is considerable. But it requires massive dosage. These nitrates are applicable only for diuresis. Very probably the renal action in nitrate diuresis<sup>67</sup> is in part a nitrite action.<sup>64</sup>

The nitrite effect of peroral nitrates depends on the reduction of nitrate in the intestinal canal. The production of nitrite is a race against the absorption of nitrate. The basic bismuth nitrates are preeminently fitted to give a prolonged and intense nitrite effect because of their relative insolubility, the practical nonabsorption of bismuth from the intestine and their slight hydrolysis. The undissolved bismuth subnitrate mixed with chyme or feces preserves a latent store of nitrate which can be but slowly dissipated. As it enters into solution bismuth subnitrate maintains a constant low concentration of nascent nitrate ions through hydrolysis. Only a small part of this free nitrate comes in contact with the intestinal wall to be absorbed but all is in intimate contact with and subject to the action of nitrate-reducing bacteria. Thus the nitrate in bismuth subnitrate is reserved in an insoluble compound, and as sparingly liberated relatively little absorbed as such, but for the most part converted into and absorbed as nitrite.

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## THE ORGANIC FRACTION OF URINARY PHOSPHORUS\*

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IN A previous paper by one of us<sup>1</sup> the question was raised concerning the amount and significance of the "organic" or "unoxidized" fraction of the phosphorus of the urine. In this present report we have attempted to establish normal values for the twenty-four hour elimination of organic phosphorus, and to study variations from the normal in a limited number of pathologic conditions.

By using twenty-four-hour collections, we have been able to secure sufficient amounts of urine to use a direct method of analysis in place of the indirect method described in the previous paper. The method which we have used and will describe is based upon the one proposed by Youngburg and Pucher<sup>2</sup> and is the same in principle. We have however radically modified the mechanics of the procedure believing that our modifications have not only slightly increased the accuracy of the method, but also simplified it and made it less time consuming.

*Procedure*—Prepare magnesia mixture by dissolving 55 gm of crystalline magnesium chloride and 70 gm of ammonium chloride in water, adding 88 cc of concentrated ammonia water (sp gr 0.90) and diluting to one liter. Let stand three days and filter before using. To 100 cc urine add 30 cc magnesia mixture. Let stand six to eighteen hours, filter through an ashless paper, using a dry funnel and receiver. Transfer 65 cc of the filtrate (equivalent to 50 cc urine) to a porcelain evaporating dish. Add 15 gm crystalline magnesium nitrate and evaporate to dryness. Ignite directly over a free flame until the residue is white and brown fumes cease to be given off. Cool, dissolve the residue in 15 to 20 per cent hydrochloric acid and again evaporate to dryness on a steam-bath. Transfer the residue to a 100 cc volumetric flask, using about 60 cc of water for the transfer. To another 100 cc volumetric flask add 5 cc standard phosphate solution (0.3509 gm pure monopotassium phosphate per liter made up in approximately tenth normal sulphuric acid. 5 cc equals 0.4 mg P) and 60 cc water. To both flasks add 10 cc Molybdate I (2.5 per cent ammonium molybdate in 5N sulphuric acid) and 4 cc of the Fiske Subbarow reducing agent (1-2-4 aminonaphtholsulphonic acid made up according to the directions given by Fiske and Subbarow)<sup>3</sup>. Make up to volume and after ten minutes read in the Duboseq colorimeter. The standard is set at 20 and the milligrams of phosphorus in the organic fraction per cc of urine is obtained by dividing 8 by the reading of the unknown solution and dividing by 50.

Blank determinations were made on the various reagents, separately and combined, and no amounts of phosphorus sufficient to give a color with the Fiske-Subbarow reagents were found.

\*From the Evans Memorial (Massachusetts Memorial Hospitals) and Boston University School of Medicine.  
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Five cubic centimeters of the standard phosphate solution was treated with magnesium nitrate and ignited, dissolved and read against an untreated 5 cc of standard phosphate solution with no gain or loss. The rather large content of the unknown in magnesium salts does not interfere with the color development.

*Normal Values*—Measurements of the twenty-four-hour output of inorganic and of organic phosphorus were made on a group of young adults, chiefly laboratory workers, all of whom were free from any signs of metabolic disease.

The results are summarized in Table I (per twenty-four hours)

TABLE I

SUBJECT	TESTS MADE	MG ORGANIC P			% OF TOTAL P		
		MAX	MIN	MEAN	MAX.	MIN	MEAN
BW male	4	10	8	9	13	0.8	12
MD male	4	11	10	11	17	1.1	14
Both males	8	11	8	10	17	0.8	13
EW female	22	11	6	8	19	1.0	13
MH female	8	13	8	9	21	0.9	12
DB female	8	11	7	9	20	0.8	13
EG female	8	12	6	9	14	0.9	12
All females	46	13	6	9	21	0.8	13
All normals	54	13	6	9	21	0.8	13

In the female subjects, the series of tests in each case covered a period corresponding to a menstrual cycle. No periodicity in the elimination of either organic or inorganic phosphorus was observed.

*Hospital Group*—This group consisted of 57 unselected cases from the endocrine diagnostic service of the Evans Memorial. The variations here were somewhat greater.

Organic phosphorus (mg per 24 hours)	Maximum	17
	Minimum	2
	Mean	8
(per cent of total P)	Maximum	2.8
	Minimum	0.6
	Mean	1.5

Only two of this group had organic phosphorus outputs exceeding the maximum of the normal group, while six were below the minimum. There being no marked correlation between the diagnosis and the organic phosphorus output in this group, we feel that it would be idle to speculate on possible causes of high and low values. Certain diseases appeared with sufficient frequency in this group, however, to enable us to decide that they were quite without effect on the organic phosphorus elimination among these may be listed thyroid failure, pituitary hypo and dysfunction, arthritis, pulmonary tuberculosis (early), and chronic infections of tonsils and nasal sinuses.

*Leucemia*—Eighteen years ago Simmons<sup>4</sup> observed an increase in organic phosphorus in the urine of a case of lymphatic leucemia. As far as we know, this investigation has not been carried any further, and we felt that it was of interest to measure the organic phosphorus output in leucemia. By the courtesy of physicians at the Boston City Hospital and the Massachusetts General Hospital,

we were able to obtain urines from three cases of leucemia, representing the three recognized types. In two of these cases we were able to make repeated observations (Table II).

TABLE II

	ORGANIC PHOSPHORUS	
	MG PER 24 HOURS	% OF TOTAL P
Case B Lymphatic leucemia	13	50
Case D Myelogenous leucemia (chronic)	20	14
	18	12
	23	17
	13	24
	12	23
	14	23
Case W Monocytic leucemia (acute)	14	17
	17	22
	14	16
	16	21
	14	22
	8	11

It will be noted that these values are definitely high, and several of them are higher than the maxima of the other series by over 30 per cent. While the magnitude of these observed changes do not correspond exactly with those observed by Symmers, his observation seems to be definitely confirmed.

## SUMMARY

1. An improved direct method for the quantitative determination of organic phosphorus in the urine is described.
2. The normal output of organic phosphorus per twenty-four hours is from 6 to 13 mg, with a mean value of 9 mg.
3. In leucemia the output is high, values up to 23 mg being observed.

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## COLLOIDAL GOLD TEST FOR POLIOMYELITIS IMMUNE BODIES IN BLOOD SERUM\*†

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IN THE study and treatment of poliomyelitis it would be desirable to have a reliable and rapid method for detecting and measuring specific antibodies and the therapeutic value of serums from human and animal sources. If this method were also applicable to the study of possible carriers and susceptibility to the disease, to the evaluation of natural and acquired immunity, and of prognosis, such a laboratory procedure might fill a recognized need.

Current procedures that require numerous monkeys for virus neutralization tests are not only costly but demand highly specialized and uncommonly difficult technique. This method in the hands of the most skillful workers is not without drawbacks and is always time consuming. Where the time element may be important, this alone is a cogent argument for a procedure that does not require weeks for definite results.

The proposed method should prove valuable for reasons that have been enumerated and particularly in selecting serums from convalescent poliomyelitis patients and from the large group of normal adults that are now known to have specific serum antibodies for poliomyelitis.

*Technic of Method*—The test to be described depends upon the well-known but perhaps little understood mechanism of the action of serum colloids in the presence of a colloidal gold sol and an electrolyte, in this instance a solution of 0.4 per cent sodium chloride. The delicacy of this test is enhanced by the addition of cholesterol to the sodium chloride, a procedure that intensifies the flocculation effect. The colloidal gold sol is prepared according to our method which has yielded a constantly uniform and dependable reagent.<sup>1</sup> The ingredients used in its preparation must be of the highest chemical purity.

Scrupulously clean glassware and syringes must be used throughout. The syringe may be rinsed with sterile distilled water prior to withdrawing the blood samples. Salt solution, citrate mixtures, or any other chemicals or preservatives must be rigidly excluded, and accurately calibrated Kahn pipettes only are to be used.

The specimens of serum, free from preservatives, are inactivated in a water-bath at 56° C for one-half hour. Serum dilutions are made with fresh doubly distilled water and a series of ten narrow test tubes (1 by 13 cm), *chemically clean* is set up containing in all but the first two tubes 0.1 c.c. of serum or its dilution as follows:

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TUBE NO	SERUM	DIST H <sub>2</sub> O	DILUTION	SERUM CONTENT
1	0.2 c.c.	—	—	0.2 c.c.
2	0.15	—	—	0.15
3	0.1	—	—	0.10
4	0.1	0.1	1:2	0.05
5	0.1 No. 4	0.1	1:4	0.025
6	0.05	0.25 Discard 0.2 c.c. of mixture	1:6	0.016
7	0.1 No. 5	0.1	1:8	0.012
8	0.05	0.45 Discard 0.3 c.c.	1:10	0.01
9	0.1 No. 8	0.1 Discard 0.1 c.c.	1:20	0.005
10	0.1 No. 7	0.3 Discard 0.3 c.c.	1:32	0.003

To all tubes are added 0.75 c.c. cholesterolized NaCl (0.4 per cent)\* and 1 c.c. Colloidal Gold Sol in the order named. The Gold Sol\*\* should be recently prepared, stabilized by three or four days of storage and not more than four weeks old and controlled in two additional tubes as follows: (1) NaCl (0.4 per cent) 0.75 c.c. and 1 c.c. Colloidal Gold should give complete precipitate in three hours; (2) NaCl (0.4 per cent), 0.75 c.c. and 2 c.c. Colloidal Gold should show no precipitate in twenty-four hours or longer. All tests with serum and each new lot of reagent are checked in this manner (Fig. 1).

Depending upon the content of antibodies, tubes will show the following reaction that is indicated by a "curve" or series of numbers, as follows:

Key	1 Complete precipitation, Rose ppt	Supernatant fluid colorless
	2 Complete precipitation, Rose ppt	Supernatant fluid pale salmon
	3 Complete precipitation, Rose ppt	Supernatant fluid salmon
	4 Partial precipitation, Rose ppt	Supernatant fluid pale rose
	5 Partial precipitation, Rose ppt	Supernatant fluid rose
Negative	(6) No precipitation, — —	Supernatant fluid pale rose
	(7) No precipitation, — —	Supernatant fluid rose (same as neg. control)

\*The NaCl solution for the test is prepared as follows: 1 gm. pure Cholesterol is added to 1 liter of 0.4 per cent sodium chloride shaken three times a day for one week and filtered.

\*\*The Colloidal Gold Sol is prepared as follows:  
A two-liter capacity Florence Flask should be used when one liter of reagent is desired and a one-liter flask for the preparation of 500 c.c. of the reagent. Frlenmeyer flasks are not satisfactory and should not be used.

1 000 c.c. double distilled water  
8 c.c. 2 per cent potassium carbonate (Merck) (S. M.)  
(Freshly prepared or not more than two weeks old)  
1 c.c. 1 per cent oxalic acid (Merck)

Particular care is necessary in heating the solution and attention is directed to the following details. The flask is placed on a piece of wire gauze having an asbestos center with a diameter of from  $3\frac{1}{4}$  to  $3\frac{3}{4}$  inches. The Bunsen burner gas flame is so adjusted as to cover the asbestos disc completely but not to extend beyond its circumference. It will be noted that the hottest part of the flame covers the entire disc with the exception of a central circular area about an inch or more in diameter. This arrangement insures correct heating and the formation of large bubbles. Heat the flask on the wire gauze rotating the flask frequently until the first large bubble appears. This usually occurs after fifteen minutes. Remove flask, rotate contents once and while the fluid is in motion add 12 c.c. of 1 per cent Gold chloride (Merck). Replace on flame and heat until five large bubbles appear. (Remove flask, rotate contents once and slowly add 5 drops (0.22 c.c.) of formaldehyde 40 per cent (C. P.) (Merck or Baker) while the fluid is still in motion. Rotate flask steadily until typical clear ruby color appears.) Promptly cover mouth of flask with a clean towel folded in several layers and moist with distilled water. Let stand undisturbed until cool.

Thermometric temperature control has proved valueless in the preparation of colloidal gold. The method described is simple and reliable. The reagent is allowed to stabilize for three or four days before use and is stored in the dark at a temperature of from 20 to 24° C. Suitable containers can be prepared by applying a coat of black lacquer to the exterior surface of the chemically clean glass stoppered bottles.

†At this point the technic has been modified with more satisfactory results as follows:  
Remove from flame and add 0.4 c.c. of a 1 to 10 dilution of 40 per cent formaldehyde (Merck's C. P.). Shake until the ruby red color that appears in two to three minutes begins to take on an eosin tinge.

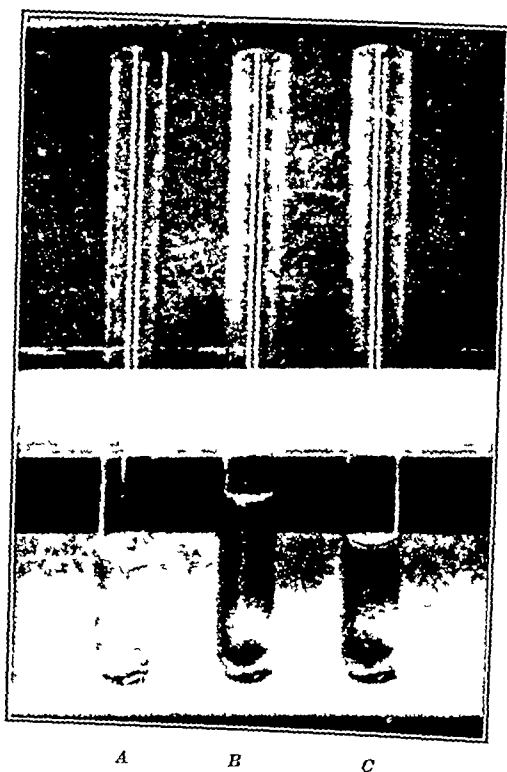


Fig 1—Control test of sensitivity of colloidal gold (24-hour reading) A One cubic centimeter of colloidal gold completely precipitated in three hours in presence of 0.75 cc cholesterolized 0.4 per cent sodium chloride solution B Two cubic centimeters colloidal gold showing no change in presence of 0.75 cc cholesterolized 0.4 per cent sodium chloride solution within or after twenty-four hours C Stock solution of colloidal gold without addition of cholesterolized sodium chloride solution

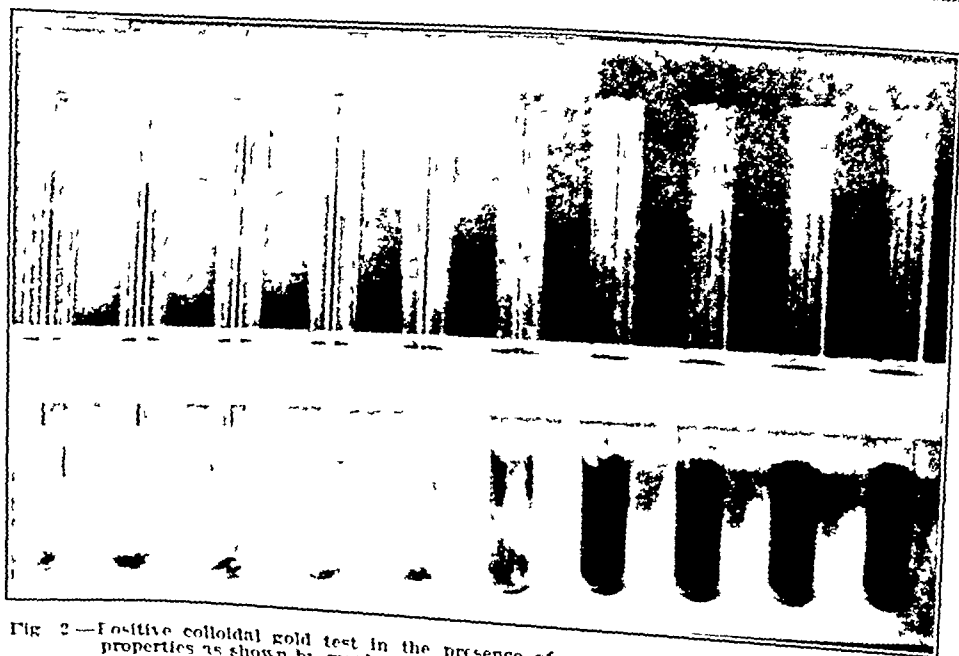


Fig 2—Positive colloidal gold test in the presence of normal adult serum having virucidal properties as shown by monkey inoculation The reaction reads 1111127777



Tubes should be kept at room temperature ( $20^{\circ}$  to  $25^{\circ}$  C) and readings can be made after three or four hours for the earliest discernible changes. Final results are read after eighteen to twenty-four hours.

A strongly positive serum with high antibody content, for example, is indicated thus 111111122 (or 1111122333). A negative serum will read 7777777777 (or 6666666666) (Figs 2-4).

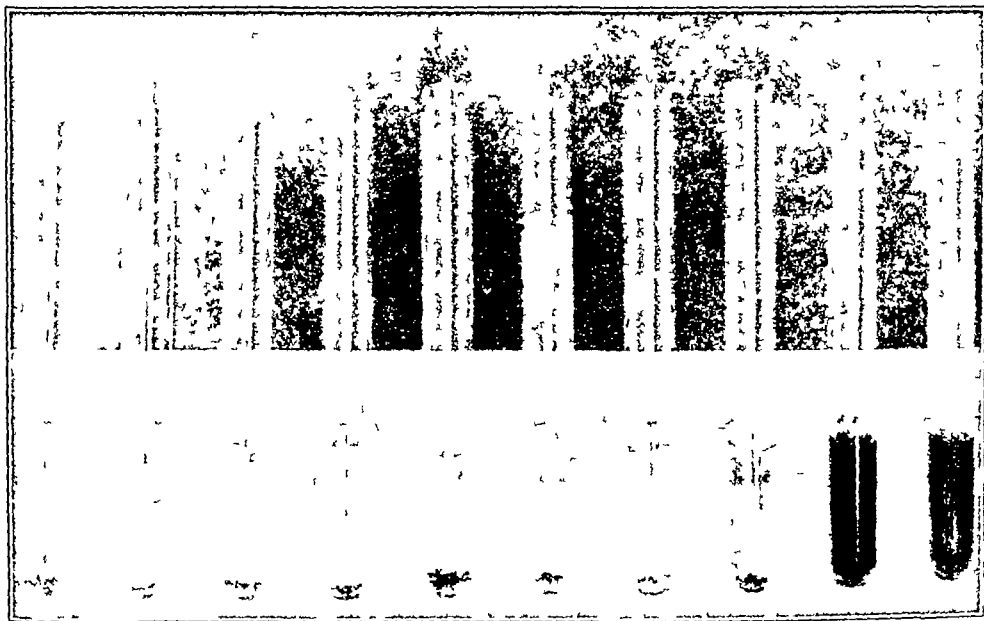


Fig 3—Positive colloidal gold test with serum from a convalescent poliomyelitis patient. The reaction reads 111111277.

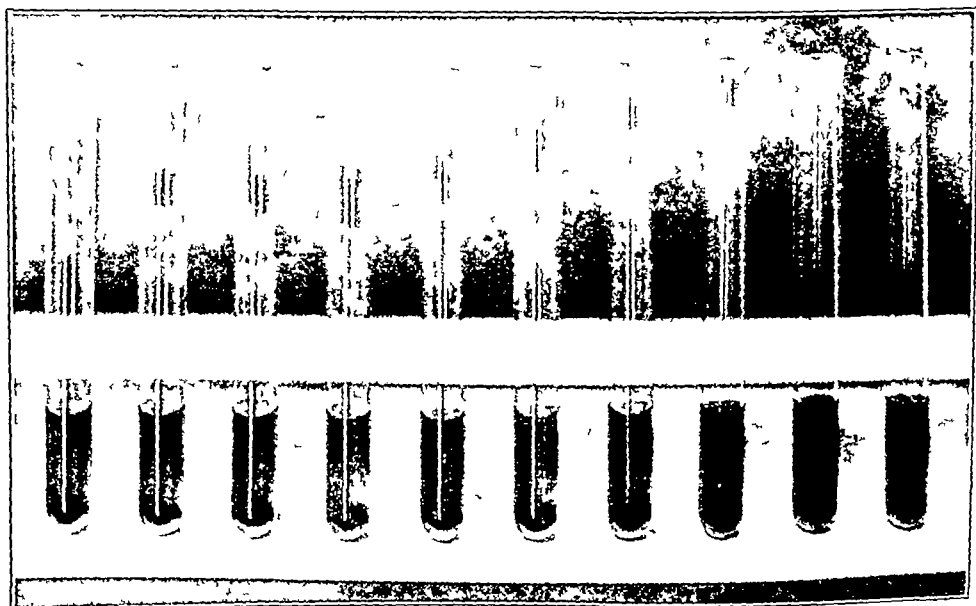


Fig 4—Colloidal gold test showing no change in the presence of a serum negative for poliomyelitis antibodies. The reaction reads 7777777777.

TABLE I

INCIDENCE OF POSITIVE AND NEGATIVE TESTS FOR ANTIBODIES IN DIFFERENT AGE GROUPS  
*Adults (Age 25-50)*

NUMBER	NEGATIVE	PER CENT	POSITIVE						TOTAL POSITIVE	PER CENT
			WEAK	PER CENT	MODERATELY STRONG	PER CENT	STRONG	PER CENT		
100	36**	36	14	14	20	20	30	30	64	64

*Children (Age 6 Weeks-19 Years)*

AGE	NUMBER	NEGATIVE	PER CENT	POSITIVE						TOTAL POSITIVE	PER CENT
				WEAK	PER CENT	MODERATELY STRONG	PER CENT	STRONG	PER CENT		
Under 1 yr (6 wk, 13 wk, 7 mo)	4	4	100.0	0	0	0	0	0	0	0	0
1-4 years	19	19	100.0	0	0	0	0	0	0	0	0
5-8 years	37	32	86.5	3	8.2	2	5.3	0	0	5	13.5
Total	60	55	91.7	3	5.0	2	3.3	0	0	5	8.3
9-14 years	35	25	71.4	2	5.7	5	14.3	3	8.6	10	28.6
15-19 years	41	31	75.6	2	4.9	3	7.3	5	12.2	10	24.4
Total	76	56	73.4	4	5.3	8	10.6	8	10.6	20	26.6
Total	136	111*	81.6	7	5.3	10	7.6	8	6.0	25	18.4

\*This group included diphtheria immune (Schick negative) 94 typhoid immunity (strongly positive Widal) 5 and pneumonia immune serums 3

\*\*This group included Schick negative as well as Widal positive

## MATERIAL STUDIED

Serums were obtained from normal and convalescent children and adults, normal actively infected, and convalescent poliomyelitis monkeys, normal rabbits, and normal and artificially immunized horses, goats and sheep. Serums were also obtained from rabbits that had been systematically immunized with different bacterial species. A high agglutinating titer was demonstrated in these serums toward the homologous organisms (Table II).

*Normal Serums—Adults and Children*—One hundred serums from supposedly normal adults without a history of poliomyelitis infection gave positive tests in 64 instances (64 per cent). The negative group included diphtheria immunes (Schick negatives) and typhoid immunes (Widal strongly positive). One hundred and thirty-six children ranging in age from six weeks to nineteen years gave negative serum reactions in 111 cases (81.6 per cent). In this negative group were in-

cluded 102 serums having demonstrable antibodies for diphtheria (94) typhoid fever (5), and pneumonia (3)

In Table I are summarized the results of tests on children and adults with reference to degree of reaction and different age groups in children

*Normal Monkey Serums*—79—Negative tests were observed in 66 (83.6 per cent) serums from these animals, and 13 were questionably slightly positive by test for antibodies in varying degrees. These positive serums were not controlled in the usual manner by virus absorption tests and the method of collecting these was not known.\*

*Normal Horse Serums*—8

*Normal Goat Serums*—4

*Normal Sheep Serums*—2

*Normal Rabbit Serums*—12

In this group of 26 serums, one was strongly positive, four moderately positive, four weakly positive, and five negative. Three negative tests out of eight were obtained with horse serum and both specimens from sheep were likewise negative, as were those from rabbits.

*Comment*—It would appear that serum antibodies may be present in certain animals that are naturally refractory or immune to infection with poliomyelitis. Stewart and Haselbauer<sup>12</sup> found normal sheep serum to be sometimes inactivating. They also reported that sheep yielding this serum when subjected to immunization with poliomyelitis virus failed to give a more potent serum. In fact, occasionally the animals lost the power of inactivation originally demonstrated by neutralization tests in the monkey.

There is other experimental evidence of a fundamental nature that has application to these matters under discussion. Buigi<sup>13</sup> tested the agglutinating power of serums of several species of animals including man, horse, dog, guinea pig, rabbit, goat, sheep, and others against different species of bacteria. It was found that these serums tended to show the same order of potency against these strains of organisms, regardless of which were tested. In further studies he demonstrated the same order of activity when the serums were tested for their property of flocculating suspensions of mastic, in principle analogous to the test with colloidal gold.

*Convalescent Serums From Children and Adults*—19—Of these, 6 were strongly positive, 7 moderately strong, 5 weakly positive, and one negative.

In all instances the degree of reaction was seemingly correlated with the clinical course of the disease as illustrated by some of the following typical clinical histories.

(Children's Hospital, San Francisco courtesy of Drs. Edward Shaw and Hulda Thelander.)

E. D., aged thirteen years. Symptoms appeared on October 6, 1930, and almost complete paralysis of both legs and right arm developed rapidly. The patient received 50 c.c. convalescent human serum after admission. On December 16, 1930, improvement was marked and the patient was in excellent condition and receiving physiotherapy. The serum, tested on this date, gave the following reaction: 2223345555.

\*As this article goes to press several articles published by C. V. Jungeblut and associates have also shown that neutralizing substances for poliomyelitis virus may be found in normal adult *rhesus monkeys* while the serum of immature animals reacts uniformly negatively. (Proc. Soc. Exp. Biol. and Med. 29: 879, 1932.)

R L, aged four years, developed symptoms and paralysis of left leg on November 10, 1930. Serum was not given. On December 16, 1930, when the patient's serum was tested, the general condition was poor and no improvement had set in. The test was negative 7777777777.

R L M, aged six years, developed symptoms and slight weakness of right leg on November 17, 1930. Fifty cubic centimeters of convalescent serum were given after admission and no progression of weakness developed. On December 16, 1930, the patient was in good condition and was receiving physiotherapy. Serum tested on this date was positive for immune bodies 2223344555.

S T, aged seven years, developed symptoms and paralysis of left arm and leg on December 14, 1930. Fifty cubic centimeters convalescent serum were given after entry into hospital. Serum for test was drawn on February 6, 1931, when the patient was convalescent, in good condition, and receiving physiotherapy. The serum was strongly positive for immune bodies 1111143333.

A C, aged seven years, had been ill for three days prior to admission into the Children's Hospital on February 21, 1931. Injections of 50 c.c. of convalescent serum had been given on the nineteenth and twentieth of February when marked weakness of the legs developed and progressed to all extremities and respiratory muscles. Transfusion of 100 c.c. of convalescent blood and injection of 100 c.c. of sheep serum were given on February 21. The diaphragm and intercostal muscles were completely paralyzed and the child was kept in the Drinker respirator almost constantly. For the past ten weeks the child has been treated in the respirator for eight to ten hours daily. The diaphragm shows virtually no movement and the arms and legs only fair recovery. At this time (May 4, 1931) the serum test was positive 1112377777.

A R, aged twelve years, admitted to hospital on March 7, 1931, had been sick eighteen hours with preparalytic symptoms. Lumbar puncture yielded cerebrospinal fluid having 31 cells, 81 per cent lymphocytes. Between March 7 and 10, 165 c.c. convalescent serum and 140 c.c. sheep serum were given. On March 12 generalized paralysis developed, six days after onset of the disease. The intercostal muscles were also involved and the child was treated in the respirator intermittently for three weeks. The response of extremities and respiratory muscles has been very satisfactory. Serum test on May 4, 1931, was positive 1112357777.

The next two cases in twins illustrate the difficulties often encountered in diagnosis, particularly when the disease occurs in the same household.

P D, aged eight years, on April 12, 1931, developed fever of 102° to 105°, photophobia, headache, vomiting, and pains in the neck and back. Slight ptosis of one eye and general hyperesthesia completed the picture on April 17, 1931, when the patient was admitted to the hospital. The cerebrospinal fluid contained 150 cells, 69 per cent lymphocytes. Forty five cubic centimeters sheep serum was given. Four days later, April 21, 1931, the temperature became normal and no weakness developed. The cerebrospinal fluid contained 84 cells with 67 per cent lymphocytes. The recovery was uneventful and a serum test was made on May 4, 1931, two weeks after improvement first set in, and was slightly positive 3335577777. June 29, eight weeks later, the test was similar 3337777777.

M D, aged eight years, twin brother of the preceding patient, developed fever, headache, and pain in the back of the neck one day after the brother became ill, and stayed in bed thirty six hours. He complained of weakness and had difficulty in walking. During the ensuing nine days until he entered the Children's Hospital on April 22, he limped from time to time when tired. Examination revealed normal reflexes and a slight toe drop on the right foot in association with a "hump." A diagnosis of probable poliomyelitis was made at this time, but no serum treatment was given to patient. A serum test made on May 4, 1931 was negative 7777777777, but on June 29, eight weeks later, the colloidal gold test was 1112233444. This definitely positive test with the serum at a time when immune substances have had an opportunity to develop suggests quite strongly that the original diagnosis of poliomyelitis was correct.

(Mount Zion Hospital, San Francisco, courtesy of Dr. Emil Jellinek.)

M G, aged twenty four, developed symptoms of weakness and gastrointestinal disturbance with marked weakness of upper extremities. One hundred cubic centimeters pooled convalescent

serum was given after admission in October, 1930. The patient improved rapidly and was convalescent after three weeks. The patient's serum was tested during the first, second, and third weeks of illness and was strongly positive for immune bodies. No paralysis developed.

A 21-year-old twenty-eight, entered the hospital in October, 1930, with profound prostration and advanced paralysis of the upper extremities. The cerebrospinal fluid showed a cell count of over 1,600 lymphocytes. Bulbar symptoms developed during the first week and paralysis extended to the accessory respiratory muscles and the lower extremities. The patient's serum was tested during the first week of illness and gave a negative reaction. 7777777777. During the fourth week, there was no change and after two months the reaction was weakly positive. 3337777777. Convalescence was protracted and after six months there was marked residual paralysis of the arms.

*Comment*—The foregoing observations in patients suggest a relationship between the clinical progress of the disease and the antibody content of the serum. The course of the infection, its duration and outcome in these examples were in close agreement with the observed tests made on the different serums obtained from these patients. In all respects the results harmonized with those obtained in experiments on monkeys.

*Convalescent and Actively Infected Monkeys*—51—Strongly positive reactions were obtained in 15, moderately strong in 12, weakly positive in 13, and negative tests in 11. Of the last named, a few of the animals showed no clinical improvement whatsoever and died, the remaining were prostrated and in poor condition at the time the serums were tested.

*Comment*—Prior to inoculation with poliomyelitis virus, all these animals gave typical negative serum reactions. Clinical improvement and progressively stronger reactions appeared during the paralytic stage and during established convalescence as the prognosis became favorable. In a number of animals subclinical doses of virus failed to cause infection but the serum test showed antibody content in varying degrees. This is in conformity with the observations made during epidemics of poliomyelitis and is also suggestive of the immunity developed by adults without knowledge of definite exposure.

For purposes of illustration and comparison a brief clinical description is given of a few of the monkeys in this experiment.

*Macacus rhesus No 3* received intracerebrally on December 5, 1930, 1 c.c. of a 5 per cent virus filtrate obtained from the Hooper Laboratories, University of California. On December 23, 1930, there was partial paralysis of the right arm and leg. On the following day, complete paralysis of the extremities on both sides had occurred. Serum tests were made eight times between December 24, 1930, and January 4, 1931, and gave entirely negative results, reading 7777777777. On January 5, 1931, the animal was moribund and etherized. Autopsy disclosed typical lesions of poliomyelitis confirmed by histologic examination of the brain and cord.

*Macacus rhesus No 4* received intracerebrally on December 22, 1930, 0.75 c.c. of a 10 per cent virus filtrate (Hooper Laboratory strain). A serum test made a few days previously was negative. 7777777777. No symptoms developed until January 28, 1931, about five weeks after inoculation, when the animal appeared somewhat slow in its movement and vomited during the day. There was some excitability and ruffled fur. No other changes were noticed and no paralysis developed. A serum test on February 17, 1931, was moderately positive. 2224567777, and at the end of February the animal was still normal in appearance and behavior. Another serum test was again positive.

*Macacus rhesus No 7* was inoculated intracerebrally on February 5, 1931, with 0.5 c.c. of a 5 per cent virus filtrate (Flexner strain). The serum test on this date was negative. 7777777777. Six days later, February 11, 1931, the animal showed slight weakness of right leg with some ataxia. February 16, 1931, on the eleventh day following inoculation, all extremities were completely par-

alyzed The serum test on February 17, 1931, was positive 1111223467 During the next two weeks there was steady improvement and the test on February 28, 1931, was strongly positive 1111112244 One month later the animal had regained the use of arms and legs to some degree and was able to hold food in its hands The serum was tested again on March 26, 1931, and gave the reading 1111122333 Toward the end of April, 1931, the animal showed progressive improvement and was able to move about fairly well

*Comment*—These observations were instructive with regard to the seeming relationship between the clinical course of experimental poliomyelitis in the monkey and in the vitio test for antibodies in the serum

Three distinct clinical features are illustrated in this experiment One animal represented a rapidly progressing poliomyelitis in which no improvement whatever occurred and was followed by death A second monkey showed only transitory symptoms of the disease that ordinarily might escape notice The third animal developed typical poliomyelitis that went on to progressive recovery These characteristics find a counterpart in the human type of infection and are in all respects similar

From the standpoint of prognosis and immune bodies in the serum the experimental results appear to be consistent The same observations had already been made with our serums from convalescent patients and in the development of serum antibodies among "normal" adults

Although more than one factor may be implicated, it would seem that the serum test for poliomyelitis antibodies confirms certain observations that have suggested mass immunization as one of the factors during epidemics and at other times As a further corollary it is clear that unrecognized infections or abortive poliomyelitis may give rise to serum antibodies that are detectable by the colloidal gold test in supposedly normal adults

*Immune Monkey Serums*—32—Tests with these serums resulted as follows 13 were strongly positive, 15 moderately strong, three weakly positive, and one negative

*Immune Horse Serums*—8

*Immune Goat Serums*—2

*Immune Sheep Serums*—2—The tests with goat serums were moderately or weakly positive and resembled normal goat serum in this respect The sheep serums yielded slightly positive reactions

Serums from artificially immunized horses (Parke and Wever) showed striking and consistently positive reactions One part (unit) of these serums had a known protective action against 2 to 10 and as high as 25 units of potent poliomye-

SERUM	POTENCY*	COLLOIDAL GOLD TEST
Polio Horse 4283	25 1 to 20 1	1111123577
Polio Horse 5268	5 1 to 20 1	1111122557
Polio Horse 4728	10 1	1111111122
Polio Horse "Tom"	20 1	1111111122
Polio Horse 5102	25 1	1115577777
Polio Horse 5259	2 1	1111235677
Polio Horse "Concentrate"	100 1	2222223345
Polio Sheep No. 117	2	1111111111
Polio Goat No. 3	2	5566677777
		5566667777

\*Expressed in terms of number of parts of virus neutralized by one part of serum as determined by intracerebral injections in *M. rhesus* monkeys

litis virus Two samples of "concentrate," effective against 100 doses of virus, also gave prompt and complete precipitation reactions Controls (ether tricesol, 0.4 per cent, to check effect of the preservative) were negative

TABLE II  
RESULTS OF COLLOIDAL GOLD TESTS WITH DIFFERENT SERUMS

SERUM	NUMBER	POSITIVE	PER CENT	NEGATIVE	PER CENT
<i>Normal</i>					
Adult	100	64	64.0	36**	36.0
Child	136	25 (e)	18.4	111*	81.6*
Monkey	79	13 (a)	16.4	66	83.6
Horse	8	5 (b)	62.5	3	37.5
Goat	4	4 (c)	100.0	0	0.0
Sheep	2	0	0.0	2	100.0
Rabbit	12	0	0.0	12	100.0
<i>Convalescent</i>					
Adult	3	3	100.0	0	0.0
Child	16	16	100.0	0	0.0
Monkey	51	39	76.5	12 (d)	23.5
<i>Immune</i>					
Monkey	32	31	96.9	1	3.1
Horse	8	8	100.0	0	0.0
Goat	2	2	100.0	0	0.0
Sheep	2	2	100.0	0	0.0
		0	0.0	10	100.0
<i>Rabbit Antiserum</i>		<p>*This group included 94 Schick negatives (diphtheria immunes), 5 typhoid immunes and 3 pneumonia immune serums</p> <p>**This group included Schick negatives and Widal positives</p> <p>(a) Serums obtained from another institution. These did not show a clean cut reaction and were not checked for specificity by means of virus absorption test</p> <p>(b) Of these four were only moderately positive</p> <p>(c) Of these, one was moderately positive and three weakly positive</p> <p>(d) These tests were made during the paralytic stage in monkeys that were not gaining and in those that died shortly afterward</p> <p>(e) Weakly positive results in four cases and moderately strong in eight (in age group nine to nineteen years). Strongly positive in eight cases (in age group fourteen to nineteen years)</p>			
Staphylococcus	2				
Streptococcus	1				
Polyvalent					
Staphylococcus and Streptococcus	1				
Typhoid	1				
Paratyphoid A	1				
Paratyphoid B	1				
Enteritidis	1				
Pneumococcus I	1				
Pneumococcus II	1				
	10				
Total	465				

*Immune Rabbit Serums*—10—These serums were obtained from a series of rabbits that had been systematically immunized with one of the following organisms: staphylococcus, streptococcus (one animal received a mixture of polyvalent streptococcus and staphylococcus), pneumococcus, and organisms of the typhoid, paratyphoid, and enteritidis group.

The colloidal gold reaction was entirely negative with this group of serums that represented antibodies for diseases other than poliomyelitis.

The results confirmed the observations also made with human serums that reacted negatively despite a high antibody content for diphtheria or typhoid fever or both.

*Comment*—The results obtained with the colloidal gold test on 465 serums and summarized in Table II, appear to warrant the conclusion that the method described is applicable to the study of poliomyelitis antibodies in serum.

The experimental findings also support certain hypotheses and some definite observations that have been made in the past and related particularly to the neutralizing power of serums from supposedly normal persons.

Our observations in this respect are in conformity with the earlier experiments of Anderson and Frost,<sup>4</sup> and Zingher,<sup>7</sup> and, more recently, Shaughnessy and associates,<sup>6</sup> Aycock and Kramer,<sup>7</sup> and Rhoads.<sup>8</sup> The inactivating power of such serums for poliomyelitis virus may equal and even surpass the neutralizing capacity of serums from persons that have recovered from an attack of the disease. This holds true for certain animal serums also, notably those derived from naturally immune or refractory species.

In the age groups including a number of infants and children up to eight years, the test has failed to demonstrate immune substances in the blood serum, except in rare instances in which weakly positive reactions occurred. This is consistent with our knowledge of susceptibility to this disease during early childhood.

Study of serums from convalescent patients and monkeys brought out facts suggesting a relationship between development of antibodies and prognosis in poliomyelitis.

In order to appreciate the general significance of the data under consideration, it may be well to formulate certain questions.<sup>9</sup> Are the normal antibodies acquired or inherited? If genetic factors are most important, will the normal antibodies appear at a certain stage of development independently of external stimuli, or is the inherited factor merely a capacity for response to a given stimulus by production of antibodies? This implies a response of some kind regardless of whether or not the environmental factor is concerned with exposure to poliomyelitis.

There are data available in other infectious diseases such as diphtheria and in the abundant studies on normal hemagglutinins and hemolysins. It is well known, for instance, that normal agglutinins are found more commonly in the serum of adult men and animals than in the young. The experiences with the Schick reaction in the case of diphtheria antitoxin are decidedly informing with respect to antibody content of serum in certain age groups. Hirszfeld<sup>10</sup> does not regard Zingher's data<sup>11</sup> on the Schick test as necessarily proving that an infection with the diphtheria bacillus was the specific stimulus. We do not know how often such a stimulus is applied in the form of infection producing no clinical symptoms.

It is conceivable that the gradual development of the antibody-producing mechanism varying with age may play a part equally as important as the chances of receiving a specific environmental stimulus. There are facts in favor of this idea. For example, the high resistance to infection with pneumococcus found in the normal fowl is associated with type specific antibodies in the blood. It would be necessary to assume that in a short space all fowls receive specific stimuli with the different antigenic elements of the pneumococcus. Similarly, it is difficult to explain the resistance of the dog, cat, and sheep and the susceptibility of the mouse, rabbit, and man to pneumococcus infection on the basis that the first named group are infected with the organism frequently and the latter only rarely. These differences



in resistance, however, are associated with variations in antibody content and the ease or difficulty with which artificial immunity may be effected

#### CONFIRMATION OF IN VITRO TEST BY IN VIVO NEUTRALIZATION EXPERIMENTS

The nature of the precipitation phenomenon exhibited by certain serums was studied more minutely by means of in vivo neutralization experiments in the monkey. It was especially desirable to evaluate the protective property of serum obtained from normal adults. As is now known such persons constitute a fairly large group having serum with demonstrable neutralizing power.

For this purpose two serums were selected from a group of normal adults, among whom over 60 per cent showed immune bodies in their serum by the in vitro test. One serum giving a negative, and the other a definite positive result with the colloidal gold test were employed.

In the experiments to be recorded, a highly potent virus obtained from the Rockefeller Institute through the courtesy of Dr. Simon Flexner was used. This virus caused typical poliomyelitis within seven days after intracerebral inoculation of 0.5 c.c. or less of a 5 per cent emulsion filtrate prepared from the brain and cord. The strain of virus had been passed through three monkeys with fairly uniform results in our hands.

The virus filtrate was mixed with 0.5 c.c. serum (previously inactivated for one half hour at 56° C), incubated at 37° C for one hour, allowed to stand overnight in the icebox (4° to 6° C), and the entire contents injected intracerebrally under ether anesthesia and careful surgical technique. Under these conditions immune serums neutralize the virus whereas strictly normal sera do not.

April 18, 1931, six monkeys were employed, two received normal, unpreserved human serum giving a negative test (7777777777), two others a human serum with a positive test (1111224477) and the remaining two received the virus alone. Table III gives the results. The control animals became paralyzed on the sixth to the eighth day, the animals that received the strictly normal serum came down on the seventh to the ninth day and the two animals receiving the positive test serum remained free of all symptoms.

*Comment*—Under the conditions of the experiment, normal adult human serum inactivated the virus only when the colloidal gold test was positive and indicated the presence of immune bodies. In this instance the serum was effective against approximately 50 to 60 M. L. D., as calculated on the usual activity of the virus.

These experiments suggested the possibility of utilizing the colloidal gold test to evaluate mixtures of serum and virus for purposes of immunization. Such mixtures of serum and virus that failed to induce poliomyelitis infection in monkeys might prove useful in developing an active immunity. One form of a crucial test would depend upon the outcome of a second inoculation in such protected monkeys with a dose of virus capable of bringing down untreated control animals. A second and more satisfactory method would be that of testing the virucidal property of serums from such monkeys. Such studies, now in progress, aim to use a quantity of virus combined with serum in appreciable excess so as to yield a positive precipitation reaction with these "protected" mixtures.

TABLE III

NEUTRALIZATION EXPERIMENTS IN MONKEYS WITH SERUMS TESTED BY COLLOIDAL GOLD METHOD

MONKEY NO	MATERIAL TESTED	AMOUNT cc	VIRUS AMOUNT cc	RESULTS
9	Unpreserved, normal adult serum giving positive colloidal gold test (1111224477)	0.5	0.5 (10% filtrate)	No symptoms, remained well
10	Unpreserved, normal adult serum giving positive colloidal gold test (1111224477)	0.5	0.62 (20% filtrate)	No symptoms, remained well
11	Unpreserved, normal adult serum giving negative colloidal gold test (7777777777)	0.5	0.5 (10% filtrate)	Typical poliomyelitis, 9 days
12	Unpreserved, normal adult serum giving negative colloidal gold test (7777777777)	0.5	0.62 (20% filtrate)	Typical poliomyelitis, 7 days
13	—	—	0.5 (10% filtrate)	Typical poliomyelitis, 8 days
14	—	—	0.5 (20% filtrate)	Typical poliomyelitis, 6 days, died on seventh day

VIRUS ABSORPTION EXPERIMENTS AND THEIR APPLICATION TO SPECIFIC SERUM POTENCY

Experiments were devised to study the possible application of the colloidal gold test to the degree of neutralization in vitro of poliomyelitis virus by specific immune serums.

Preliminary attempts were made by combining virus filtrate as a serum diluent before addition of the gold sol and electrolyte. The effect was to prevent precipitation and the serums behaved in this respect like normal negative monkey serum.

The method adopted for quantitative study utilized a number of our immune monkey serums and potent antipoliomyelitis horse serums obtained through the kindness of Dr. William H. Park, New York City Department of Health.<sup>12</sup> Five and 10 per cent virus emulsions (Flexner strain) were prepared with doubly distilled water and centrifuged for ten minutes at 3,000 revolutions per minute. Dilutions of virus were made from the supernatant fluid and distributed in a series of test tubes. To these was added next to the least amount of serum found by previous tests to cause complete precipitation of the colloidal gold according to the regular method. After one hour incubation at 37.5° C. the colloidal gold and sodium chloride solution were added and the results read twenty-four hours later. Controls included normal serum, poliomyelitis serum in the same amounts without virus, and dilutions of encephalitis (Levaditi) and herpes virus (Goodpasture) obtained through the kindness of Dr. Schultz of Stanford University.

The effects of adding virus to serums were studied in the following manner (1) Different concentrations of virus in fixed amounts were combined with varying dilutions of serum (2) Different dilutions of a 5 per cent or 10 per cent virus in fixed amounts were combined with fixed amounts of serum

The following protocols illustrate typical experiments and are summarized in Tables IV and V The double barred line indicates the transition from definite precipitation (complete or partial) to absence of precipitation (slightly positive to completely negative zones)

TABLE IV  
VIRUS ABSORPTION TESTS AND EFFECT ON COLLOIDAL GOLD REACTION

SERUM (A) CC	VIRUS (A) CC	NONE	5%	10%	20%	NORMAL SERUM
0.2	0.05	2	2	<u>3</u>	7	7
0.15	0.05	2	2	7	7	7
0.1	0.05	2	<u>2</u>	7	7	7
0.1 (1:2)	0.05	2	7	7	7	7
0.1 (1:4)	0.05	2	7	7	7	7
0.1 (1:6)	0.05	2	7	7	7	7
0.1 (1:8)	0.05	2	7	7	7	7
0.1 (1:10)	0.05	2	7	7	7	7
0.1 (1:20)	0.05	2	7	7	7	7
0.1 (1:32)	0.05	3	7	7	7	7

(a) Serum from convalescent M. rhesus No. 7 and poliomyelitis virus (Flexner strain)

TABLE V  
EFFECT OF VIRUS ABSORPTION ON COLLOIDAL GOLD REACTION, USING POLIOMYELITIS VIRUS AND OTHER VIRUSES COMBINED WITH POLIOMYELITIS IMMUNE AND NORMAL SERUM

SERUM (A) CC	5% VIRUS (A) CC	NONE POLIO NORMAL		AFTER ABSORPTION WITH VIRUS					
				POLIOMYELITIS POLIO NORMAL		HERPES POLIO NORMAL		ENCEPHALITIS POLIO NORMAL	
0.2	0.05	2	7	2	7	2	7	2	7
0.15	0.05	2	7	2	7	2	7	2	7
0.1	0.05	2	7	<u>2</u>	7	2	7	2	7
0.1 (1:2)	0.05	2	7	7	7	2	7	2	7
0.1 (1:4)	0.05	2	7	7	7	2	7	2	7
0.1 (1:6)	0.05	2	7	7	7	2	7	3	7
0.1 (1:8)	0.05	2	7	7	7	3	7	3	7
0.1 (1:10)	0.05	2	7	7	7	3	7	3	7
0.1 (1:20)	0.05	2	7	7	7	3	7	3	7
0.1 (1:32)	0.05	2	7	7	7	3	7	3	7

(a) Serum from convalescent M. rhesus No. 7 poliomyelitis virus (Flexner strain) and herpes (Goodpasture strain) and encephalitis virus (Levaditi strain)

In the following experiments illustrated by specimen protocols in Tables VI and VII virus absorption tests were applied to serums of different potencies The technique differed somewhat from the preceding series of tests in that the cholesterolized sodium chloride solution (0.4 per cent) was added along with the colloidal gold to the previously incubated mixtures of serums and different viruses This

method was found better suited to sharper end-points in the reading of the absorption tests, and this procedure was adopted

*Comment*—These observations indicate that the precipitation of colloidal gold by immune serums does not occur in the presence of poliomyelitis virus. The phenomenon is apparently dependent upon the amount of virus used for absorption and the antibody content of the serums in question. By varying the concentration of one or the other, singly, the reversal of reaction can be demonstrated (Tables IV, V, and Va)

TABLE VA

EFFECT OF VIRUS ABSORPTION ON COLLOIDAL GOLD REACTION, USING POLIOMYELITIS VIRUS COMBINED WITH VARYING AMOUNTS OF CONVALESCENT POLIOMYELITIS SERUM

(A) SERUM cc	VIRUS (10%) cc	REACTION (B)	
		BEFORE ABSORPTION	AFTER ABSORPTION
0.05	0.2	1	5
0.05	0.15	1	1
0.05	0.1	1	1
0.05	0.1 (1:2) (0.05)	1	1
0.05	0.1 (1:4) (0.025)	1	1
0.05	0.1 (1:6) (0.016)	1	1
0.05	0.1 (1:8) (0.012)	1	1
0.05	0.1 (1:10) (0.01)	1	1
0.05	0.1 (1:20) (0.005)	1	1
0.05	0.1 (1:32) (0.003)	1	1
0.03	0.2	1	7
0.03	0.15	1	3
0.03	0.1	1	3
0.03	0.1 (1:2) (0.05)	1	2
0.03	0.1 (1:4) (0.025)	1	2
0.03	0.1 (1:6) (0.016)	1	2
0.03	0.1 (1:8) (0.012)	1	1
0.03	0.1 (1:10) (0.01)	1	1
0.03	0.1 (1:20) (0.005)	1	1
0.03	0.1 (1:32) (0.003)	1	1
0.02	0.2	1	7
0.02	0.15	1	7
0.02	0.1	1	7
0.02	0.1 (1:2) (0.05)	1	3
0.02	0.1 (1:4) (0.025)	1	3
0.02	0.1 (1:6) (0.016)	1	3
0.02	0.1 (1:8) (0.012)	1	3
0.02	0.1 (1:10) (0.01)	1	2
0.02	0.1 (1:20) (0.005)	1	1
0.02	0.1 (1:32) (0.003)	1	1

(a) The serum from an adult female patient that had recovered fully from a severe attack of poliomyelitis was highly potent and small amounts neutralized 0.5 cc of a 5 to 10 per cent filtrate of an active Flexner strain of virus

(b) Note the increased precipitation effect as the amount of virus is diminished and the wider range of more complete precipitation in the presence of greater excess of antibodies. In this and in all other tables the colloidal gold reaction is indicated by numbers as described in the text

The underlying mechanism of this phenomenon and the principle involved lend themselves to experimental study in various ways. It seems likely that when the antibody is in excess due to incomplete absorption the serum will still give a

TABLE VI

VIRUS ABSORPTION TESTS AND COLLOIDAL GOLD REACTION WITH VARIOUS ANTIPOLIOMYELITIS HORSE SERUMS

A AFTER VIRUS ABSORPTION		B BEFORE VIRUS ABSORPTION				
SERUM (A) C C	VIRUS (10%) (1) C C	POLIOMYELITIS IMMUNE SERUMS				
		4283 A B	4728 A B	"TOM" A B	5102 A B	5259 A B
0.05	0.00	1 1	1 1	1 1	2 1	2 2
0.05	0.15	4 1	3 1	5 1	5 1	5 2
0.05	0.1	4 1	3 1	5 1	5 1	4 2
0.05	0.1 (1.2)	3 1	2 1	5 5	4 1	3 2
0.05	0.1 (1.4)	2 1	1 1	1 5	3 2	2 2
0.05	0.1 (1.6)	1 2	1 1	1 7	3 3	2 2
0.05	0.1 (1.8)	1 3	1 1	1 7	3 5	2 3
0.05	0.1 (1.10)	1 5	1 1	1 7	3 6	2 3
0.05	0.1 (1.20)	1 7	1 2	1 7	2 7	2 4
0.05	0.1 (1.32)	1 7	1 2	1 7	2 7	2 5

(a) Serum from poliomyelitis horses (Park and Weaver) and poliomyelitis virus (Flexner strain)

TABLE VII

VIRUS ABSORPTION TESTS WITH DIFFERENT VIRUSES AND POLIOMYELITIS IMMUNE SERUMS

CONVALESCENT SERUM (20 1)			10% VIRUS (1) C C	SERUM (1) C C	HORSE SERUM (2 1)		
POLIOMYELITIS	ENCEPHALITIS	HERPES			POLIO	ENCEPH	HERPES
3	3	3	0.2	0.05	7	2	3
3	3	3	0.15	0.05	7	2	3
2	3	3	0.1	0.05	7	2	2
2	3	3	0.1 (1.2)	0.05	6	2	2
2	3	3	0.1 (1.4)	0.05	3	2	2
2	3	3	0.1 (1.6)	0.05	3	2	3
2	3	3	0.1 (1.8)	0.05	2	-	2
2	3	3	0.1 (1.10)	0.05	2	-	2
2	3	3	0.1 (1.20)	0.05	2	-	2
2	3	3	0.0	0.05	2	-	2

(a) Human convalescent serum, horse serum (immune 5259), poliomyelitis virus (Flexner), herpes (Goodpasture) and encephalitis viruses (Levydit)

positive precipitation test. The factors that modify the outcome of this absorption are doubtless the amount of virus on the one hand and, on the other, the potency of the serum. If this be correct the effective neutralizing power of a given serum as demonstrated by this method ought to be shown by the result of intracerebral injections of suitable mixtures into *Macaca mulatta* monkeys.

Preliminary experiments so devised have already borne out this hypothesis. For example, serum-virus mixtures corresponding to the contents of tubes showing definite precipitation (reading "1") and those giving definitely negative tests (reading "7") in an absorption series were inoculated into monkeys. The results, although purely tentative, indicate the presence of adequate protective antibodies

in serum-virus mixtures that give a positive precipitation test, whereas typical poliomyelitis results from injection of mixtures yielding negative tests in vitro

The procedure was essentially as follows. Mixtures of given serums, serially diluted, and 10 per cent virus suspensions were subjected to the colloidal gold test. Combinations were selected according to the presence or absence of positive precipitation indicative of "protected" or "unprotected" mixtures, respectively. The material employed for intracerebral injection into macacus rhesus monkeys was uniformly made up to a volume of 0.8 c.c. Suitable controls included mixtures of strictly normal serum and virus and additional virus controls combined with physiologic salt solution only. The strain of active virus used had been preserved in 50 per cent glycerol for seven months.

The results are summarized in Table VIII.

Pertinent to this discussion is the possible effect of adsorbing bodies on the inactivating substances in serum. Rhoads<sup>13</sup> has already shown that suspensions of normal monkey brain are entirely devoid of the power to remove the inactivating

TABLE VIII

NEUTRALIZATION EXPERIMENTS IN MONKEYS WITH "PROTECTED" MIXTURES OF SERUM AND VIRUS TESTED BY COLLOIDAL GOLD METHOD

M RHEUS NO	MATERIAL TESTED	AMOUNT C C	VIRUS AMOUNT C C	RESULTS
5	Convalescent adult serum giving positive colloidal gold test ("protected" mixture)	0.46	0.2	No symptoms, remained well
15	Physiologic salt solution	0.6	0.2	Typical poliomyelitis, sixth day, died on seventh day
16	Convalescent adult serum giving positive colloidal gold test ("protected" mixture)	0.3	0.2	No symptoms, remained well
21	Convalescent adult serum giving negative colloidal gold test ("unprotected" mixture)	0.02	0.2	Typical poliomyelitis, twelfth day, complete paralysis
22	Convalescent adult serum giving negative colloidal gold test ("unprotected" mixture)	0.04	0.2	Typical poliomyelitis, prostrate eighth day
24	Convalescent adult serum giving positive colloidal gold test ("protected" mixture)	0.6	0.2	No symptoms, remained well
25	Physiologic salt solution	0.6	0.2	Typical poliomyelitis, tenth day, prostrate eleventh day
26	Normal adult serum giving negative colloidal gold test (no ppt)	0.6	0.2	Typical poliomyelitis, eleventh day, prostrate thirteenth day

substances contained in antipoliomyelitis horse serum. In our experiments this possibility was also anticipated and controlled by means of absorption tests with encephalitis and herpes virus filtrate and normal brain filtrates and centrifugalized emulsions. The absorption effects appeared to be specific for poliomyelitis virus only.

Further studies are suggested here in relation to the phenomena of dissociation of virus in serum-virus mixtures, the possible effects of quantitative alterations in globulins and serum proteins, and other problems bearing upon physiochemical factors. The possibility is also suggested of using the colloidal gold test in the selection of "protected" mixtures of poliomyelitis immune serum combined with virus for purposes of active immunization.

#### DISCUSSION

We do not wish to be misunderstood as assuming that the colloidal gold test described here is absolutely final. The studies are by no means completed and will bear repetition over a period of time. As far as the results have gone they appear to be convincing. To the extent that in an experimental study of this type a certain method of approach must be followed, the results are self-explanatory. The materials selected with this end in view have yielded data that are not inconsistent.

The use of convalescent serum for the treatment of poliomyelitis has led to search for suitable serums from other sources. A large percentage of supposedly normal adults has been found satisfactory in this regard. It is interesting that very young children and infants do not show such neutralizing power of their serum. This is in accord with the facts of greater susceptibility to the disease and is in keeping with the general principles of immunity to infections. A striking analogy is found in diphtheria, for which the Schick test has shown decreasing susceptibility in the higher age groups beyond that of six years.

Attention should be called to the results noted in a group of thirteen out of sixteen normal monkey serums obtained from another institution\*. The observations must stand, but an explanation for this discrepancy must wait upon additional demonstrations of the virus-neutralizing power of such serums tested in the monkey as well as upon the technique used in collecting and storing these serums. The colloidal gold test is supposedly an *indicator* of antibodies in the serum without reference to the absolute neutralizing power of serums so tested. There are often experimental discrepancies between *in vivo* and *in vitro* inactivating power. The inference is that there may be quantitative variations in immune capacity. Also a human being or animal may yield a neutralizing serum without being adequately protected against a potent strain of virus.

Where antibodies have been demonstrated in the serum of supposedly normal adults or irregularly in animals such as the monkey, the burden of proof must rest on data showing that such "normal" serum is actually protective in the presence of virus. This does not apply in the same degree to refractory animals such as the horse, goat, or sheep in which the disease cannot be reproduced.

We believe that our results with an *in vitro* method of demonstrating serum antibodies for poliomyelitis are in reasonably good agreement with the clinical and experimental material studied.

\*See footnote p. 172

The quantitative relationships of serum potency to effective neutralization of virus by animal test have been corroborated by virus absorption technique in vitro with analogous results

#### SUMMARY AND CONCLUSIONS

The in vitro colloidal gold test for serum antibodies in poliomyelitis has been applied to 465 different serums from human and animal sources. The material used represented all stages of the infection as well as normal and convalescent individuals.

Experiments have demonstrated the value of the method by comparing the serum tests with the results of in vivo neutralization tests with the same serums in *M. rhesus* monkeys.

The specificity of the test and its quantitative aspects have been controlled by means of detailed absorption experiments employing poliomyelitis virus with encephalitis and herpes viruses and further comparison with results obtained with human and animal serums having antibodies for diseases other than poliomyelitis.

More than 60 per cent of 100 normal adults and only 18.4 per cent of the 136 normal children tested showed immune substances in the serum. In the last named group, only 8.3 per cent of the serums in the age group of one year or under and eight years were positive, 5 per cent weak, 3.3 per cent moderately strong, and 26.6 per cent of the children between the ages of nine and nineteen years gave positive reactions, of which 10.6 per cent were moderately strong and 10.6 per cent strongly positive. In the adult group (ages twenty-five to fifty years), 30 per cent gave strongly positive and 20 per cent moderately strong reactions. Among 111 negatively reacting sera obtained from children were included 94 diphtheria immunes, 5 typhoid immunes, and 3 pneumonia immunes. The group of 36 negatively reacting adult serums likewise included Schick negatives and strongly positive Widal tests. Antibodies for diseases other than poliomyelitis did not give false positive reactions. This was shown further in tests with serums from immunized rabbits (Tables I and II).

A colloidal gold test has been devised for the detection of immune substances against poliomyelitis in the blood serum. The application of the method in the study of poliomyelitis has been shown to have a direct bearing and practical value in the following problems:

- 1 Study of susceptibility to this disease among the general population
- 2 Selection of donors' serum for therapeutic use in poliomyelitis, especially during outbreaks
- 3 Evaluation of therapeutic potency of serums from human and animal sources
- 4 Prognosis during the course of poliomyelitis as related to the progressive development or complete absence of serum antibodies
- 5 In vitro selection of "protected" mixtures of poliomyelitis immune serum combined with virus for purposes of active immunization

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# SEPTICEMIA DUE TO HIGHER BACTERIA\*

## CASE REPORT AND BACTERIOLOGIC FINDINGS

SARA ALICIA SCUDDER, B A , NEW YORK, N Y

A GROUP of higher bacteria was reported by Scudder and Belding<sup>1</sup> (1929) from cases of urethritis and cervicitis, associated with other organisms in cases of chronic infection purported to be of gonorrheal origin. The organisms have since been found in many instances associated with others in the genitourinary and respiratory tracts (Case reports from the Bellevue-Yorkville Health Demonstration (1930)<sup>2</sup> and from the City Hospital, Welfare Island, N Y). Concrete examples may be cited: labial ulcer in a child with Vincent's organisms, tuberculosis of the parotid, E. friedlander pneumonia. In the last instance *Encapsulatus friedlander* invaded the blood stream and carried in its wake a mixed flora including higher bacteria and *N. catarrhalis*. Comparative experiments were carried out with similar strains from the respiratory tract and with the streptococcus-pneumococcus types for purpose of identification. Nomenclature for this type of organism has not been established, although it shows consistent morphologic similarity to streptothricaceae. Cumulative evidence, however, points to generic relationship to mycobacteriaceae.<sup>3</sup>

Proof of pathogenicity of this type of higher bacterium has been sought since 1927. The present case of rheumatic heart disease which was studied during the summer of 1931 showed no other type of organism in blood culture. Evidence of focal infection in other tissues which might have prepared the way for blood stream invasion could not be proved.

### CASE HISTORY

The patient, a white male, was admitted to City Hospital, service of Dr. E. P. Shelby, on June 12, 1931 with a classical history of rheumatic fever. Tonsillectomy had been performed at six years for hypertrophy. In his eighth summer he had "growing pains" and at sixteen to nineteen years repeated attacks of sore throat. At this time cardiac disease was discovered during physical examination for insurance. At twenty-two and twenty-five years respectively there occurred repeated attacks of rheumatic fever with joint involvement. The last illness began at the age of twenty-six and progressed with moderate severity for one year, then very rapidly for the last three months, presenting symptoms of embolic processes. Autopsy was not obtained.

### BACTERIOLOGY

A series of blood cultures, June 16, 1931 to July 13, 1931, showed higher bacteria: flocculent growth in broth, radiate colonies in semisolid agar, discrete mold-like and pea-sized colonies in the original blood culture. The colony in blood agar was of the viridans type, although mildly hemolytic in first culture. The number of organisms were about two per cubic centimeter in the first culture and about

\*From the Pathologic Laboratory, City Hospital, Department of Hospitals.  
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twenty per cubic centimeter in the final culture. Transfer from discrete colonies to small tubes of broth gave radiate or tuft-like colonies subtended by long trailing filaments which reached to the bottom of the tube. Settling of this orientation of growth took place upon slight agitation (Fig 1).

The following biochemical tests do not aid in identification of the organism: bile solubility, aesculin fermentation or methyl red test. The simplest means of identification are to be found in their radiate colony formation in broth or semi-solid agar, and the study of hanging drop with oil immersion lens for observation

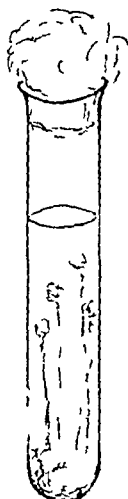


Fig 1—Culture in eighteen hours showing long filaments prior to sedimentation

of filamentous morphology. Serologic differentiation may be shown of value. Detailed report of biochemical and serologic characteristics will be published later in a study of streptococci isolated from cases of rheumatic fever.

#### SUMMARY

An instance is reported of isolation of a higher bacterium from the blood stream in a case of chronic rheumatic heart disease. The organism is most likely a secondary invader and the direct cause of the terminal clinical features.

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# LABORATORY METHODS

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## THE CARE, BREEDING AND WEIGHT CHANGES OF RABBITS IN THE LABORATORY\*

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FOR the past two years the author has been engaged in the breeding of rabbits under laboratory conditions. The purpose of this breeding has been to establish a strain of rabbits, the heredity of which would be well known before attempting any experiments upon the transmission of acquired characteristics. During this time numerous visitors have commented upon the possibility of raising rabbits in rather limited space and under indoor conditions. As a result of these comments, it seemed worth while to collect the information for publication. It is the purpose of this report, therefore, to summarize the information that has been gathered on the care, breeding, and natural history of rabbits under laboratory conditions.<sup>1</sup>

*Breeding Space and Cages*—The room occupied by the animals being bred is 14 by 13 feet. The walls are brick and the floor is waterproofed cement with a drain connection. Two large windows furnish light and ventilation. Fig 1, roughly drawn to a scale of  $\frac{1}{4}$  inch to the foot, shows the arrangement of the cages.

The animals too young to breed and excess stock are kept outdoors on a ledge surrounding the breeding floor, in two pens, each of which is 12 by 4 feet. The males are kept in one pen and the females in the other. These pens have tile floors with drains. In each pen there is a waterproofed box large enough to hold the food containers. Other than this the animals have no shelter.

The cages in the breeding room are of two types and sizes. The smaller cages are used for housing the males, one animal to a cage. They are 20 by 19½ by 15 inches and made of galvanized iron and wire. Attached to one side of the cage is a hay rack and in the bottom is a removable tray. Food (oats) is kept in galvanized iron cups of the reservoir type, attached to the door by a bolt and nut. The water cup is attached so that the water is entirely outside of the cage. On the outside of the door there is a cardholder in which can be inserted a 3 by 5 inch card (see Fig 2).

The large cages are used for housing the females and their young. They are 36 by 32 by 18 inches. They differ from the smaller cages in not having a removable pan and the water supply is contained in a poultry-type reservoir.

\*From the Wilmer Institute of Ophthalmology, The Johns Hopkins University.  
Received for publication March 20, 1922.

<sup>1</sup>This work was begun after a visit to the laboratories of Dr. M. F. Guyer at the University of Wisconsin and some of the information herein reported was obtained from Dr. Guyer and his associates.

In these cages, to prevent the spilling of bedding, the front edge of the floor has been flanged to a height of one inch (see Fig. 3)

*General Care of Cages*—The cages are cleaned by scraping and brushing the floor twice a week. At least once every two weeks, the entire cage is dropped into a vat of boiling water for about five minutes. The floor of the room is swept

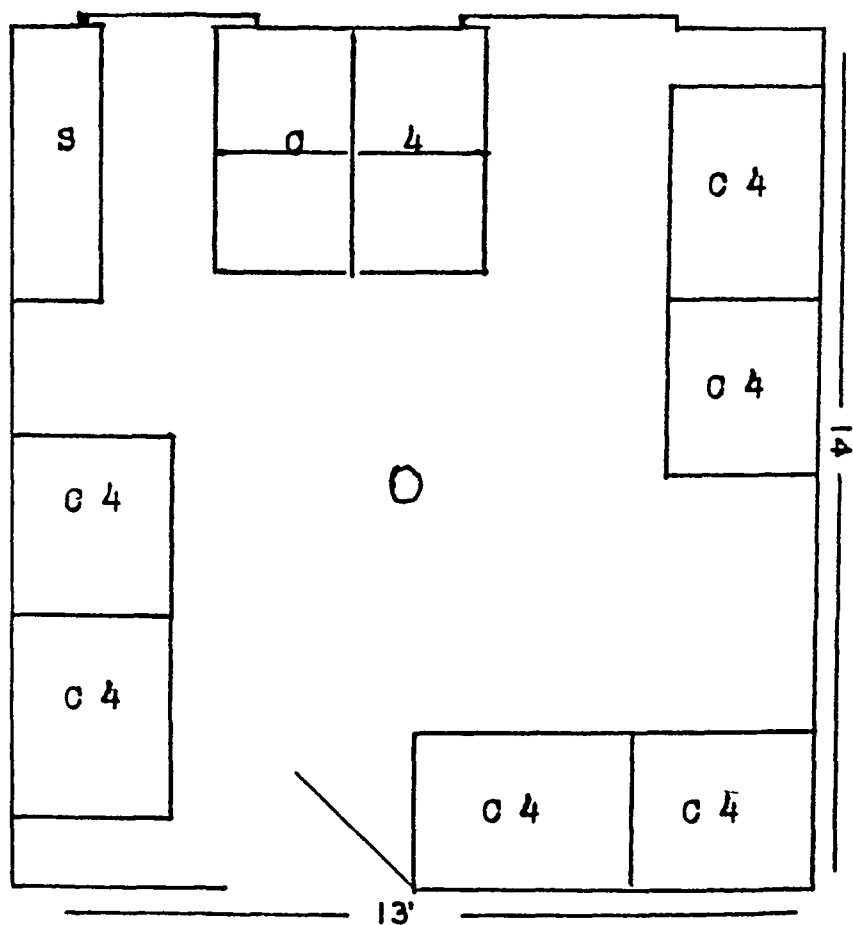


Diagram I

S - sink      C - cage      4 - tier of 4

Fig 1

at least once daily and is washed with water and a commercial disinfectant at least twice a week. The outdoor pens are washed daily with water during warm weather and as often as the weather permits in cold weather. After each cleaning the floors of the indoor cages are well covered with fresh shavings.

With a cleaning program such as the above no roaches or bedbugs are to be found anywhere on the floor occupied by the animals. During the time this regime has been in operation there have been sporadic and mild cases of snuffles

but no epidemics. Such deaths as have occurred have been mainly in the outdoor pens following heavy rainfalls or periods of intense cold.

*Food*—Each cage contains a hay rack which is filled daily with clover and alfalfa hay. Each cage is furnished with a cup of oats daily. Mother and young are fed rolled oats until they are separated. Each adult receives daily a handful of greens in the form of lettuce or cabbage leaves, or beet tops discarded from the hospital kitchens. Animals being weaned are given greens sparingly and the supply is placed in the cage where the greater portion is inaccessible to the young. It has not been necessary to supplement the mother's rations at term to prevent her from killing her young.

*Breeding*—The rabbit reaches sexual maturity about the sixth month. At this time the mature animals are moved from the large pens to the breeding cages. At least one week is allowed for the animal to adjust itself to its new surround-

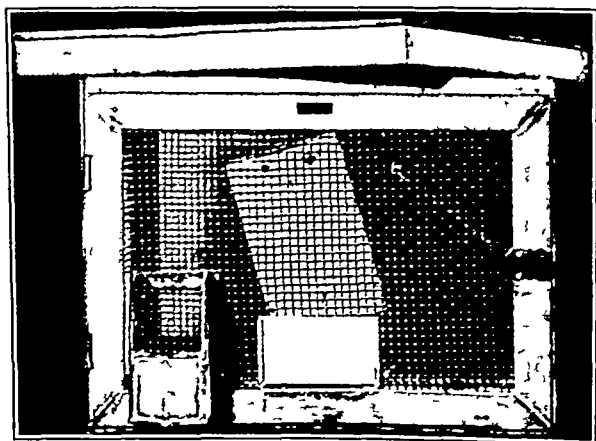


Fig 2

ings. The female is then placed in the male's cage. They are watched for a few minutes and if they do not fight they are kept together for twenty-four hours. The female is then returned to the large cage. It is important that this order of events be followed for several reasons. First if the male is placed in the female's cage he may show more interest in his new surroundings than in the female and in many instances pregnancy does not result if the male is removed in twenty-four hours. Second, if the male shows the proper amatory spirit the female may be disinterested and the larger cage gives her more space to avoid the male.

At the end of the third week the small breeding box is placed in the female's cage. (See Figs 3 and 4.) This box is made of wood and has one side that turns down so that the interior of the box can be seen without disturbing the young. The mother's entrance is purposely made small so that when she leaves the nest any nursing young clinging to her will be torn loose and not dragged out into the cage where they usually die from neglect.

Sometime during the latter part of the fourth week, the female carries hay into the breeding box and begins pulling fur to make a nest. On the thirtieth or thirty-first day after removal from the male's cage the litter is born. The young

are perfectly helpless until the tenth day when the eyes begin to open. During this period it is inadvisable to handle the young except with forceps and then only to remove dead ones if the mother does not do this. Handling of the newborn with naked hands usually results in the mother either killing or neglecting her litter. If the above routine is followed the mother will seldom kill her young, and then usually only the first litter which in any case is small, four to six as compared with eight to ten in the later pregnancies.

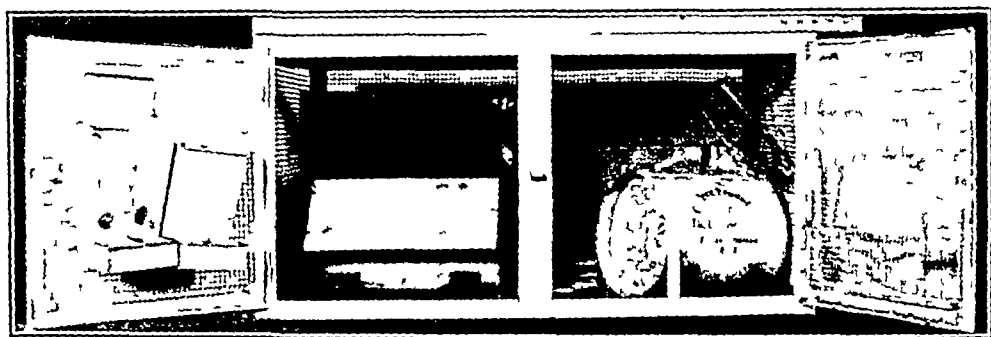


Fig 3

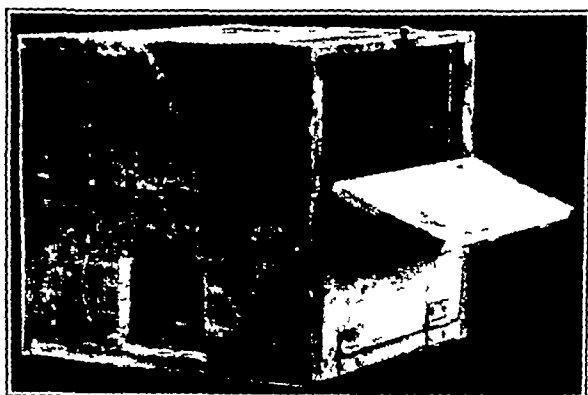


Fig 4

On several occasions litters, aged two to five days, have been handled with no ensuing deaths in the following manner. The nest, cage, and mother were sprinkled with strong smelling talcum powder. On the following day the young were handled with rubber gloves that had been sprinkled with the same powder and sterilized in the autoclave. Using this method it has been possible to make subcutaneous injections of various substances which in themselves are not lethal.

A rabbit can be remated within the first week after parturition. If this is done the litter should be weaned about the third week. Such early weaning, however, retards the weight gain and such animals are slow in reaching weight maturity. If several animals litter at the same time and if for any reason early remating is desirable in one of the group the young can be moved singly or in pairs daily to the foster-mother's nest using forceps to handle the young. This

method allows for a slow decrease in the mother's milk supply and places a gradually increased burden on the foster-mother

Females that are mated frequently are better breeders than those that are mated infrequently and that have gotten overweight. The female rabbit is generally considered to be perpetually in heat. Under cage conditions in Baltimore, however, there is a seasonal variation. From June to October a litter is a rare occurrence. If any young are born, the number is less than usual and they rarely attain maturity. In foretelling successful matings, a dusky, red, swollen appearance of the vaginal mucosa usually indicates a ready acceptance of the buck, but this is by no means a sure sign of a litter. If for any reason it is desirable to experiment on rabbits during pregnancy it is best to use animals that have littered and brought their young to the weaning period successfully on two previous occasions.

The young rabbits are usually kept with the mother for six weeks. It is difficult to differentiate the sexes before the end of the sixth week, but at this time, they are completely weaned and the sexes readily differentiated. They are identified by sex, color markings, and any gross deviations from the normal and are then tagged.

The tags are aluminum bands ordinarily used for pigeon legs. The proper number is stamped on the tag and the tag is inserted into a slit cut by a knife-edge punch parallel with the long axis of the ear and medial to the marginal ear vein. If the cut is made about 1 cm. from the ear margin, and the tag properly closed, the animal rarely loses the marker. The males are tagged in the left ear, the females in the right.

The breeding of large numbers of rabbits and the keeping of proper records demands as simple a system of record keeping as possible, but which is still complete enough to be worth while. In this study we have attempted to keep a record of the ancestry, coat markings, gross deviations from normal, conditions of the eyes, and weight changes, particularly those associated with any pathologic states.

The method of numbering is one commonly used by geneticists while the record cards have been devised by me. All animals purchased from a dealer are given a serial number. When two such animals are mated, the resulting litter is given a symbol. An example is given.

Female 21 × Male 5 = U5A

Individuals in this litter

become U5A 1

U5A 2

U5A 3 and so on

Female U5A 4 × Male U5A 1 = U33B

U = untreated, referring to the experimental procedures on the parents

5 = the fifth pair of animals mated

A = the first time this particular pair of animals have been mated

U = as above

33 = the thirty third pair of animals mated

B = the second time this pair of animals has been mated

To record the matings, Form I is used. To record the litters, Form II is used. To record the weights, markings, and experimental procedures, Forms III and IV are used. They are 3 by 5 inches in size and the first three are printed on bond paper. Form IV for use as a self explanatory and typical records is shown.



*Results*—A pair of rabbits was mated in December, 1929. The first litter was born in January, 1930. The brothers and sisters in this litter were first interbred in September, 1930. Mating brother and sister almost exclusively, fifty-four matings were done. Thirty litters were actually born. Of these four litters were destroyed by the mothers. The others were successfully weaned. These litters contained one hundred and twenty-one individuals. Of these eighty-three are now living and at least three months old. Twelve died as a result of experimental procedures. Ten died following exposure to a severe storm. Sixteen died at varying intervals before attaining an age of six months, from unknown causes. Autopsies performed on the majority of the last group showed nothing but emaciation.

*Weight Changes in Rabbits*—While it is rather a common laboratory custom to record the weight changes of experimental animals, there have been few reports published dealing specifically with this subject. Pearce and Van Allen<sup>1</sup> have reported on the gross and organ weight changes in rabbits exposed to various degrees of lighting. Their results suggest that the effect of various environments is reflected in the animal's weight.

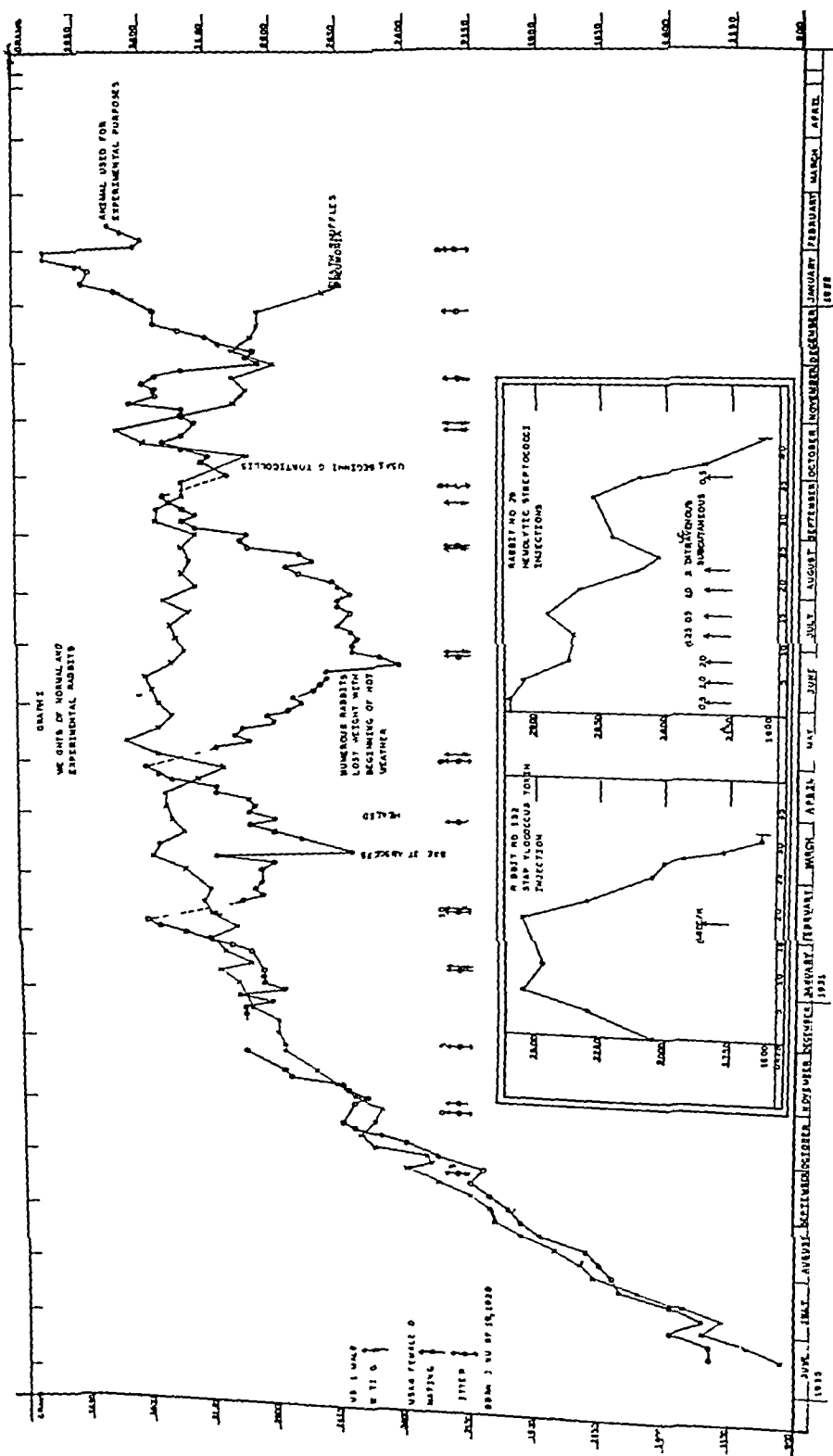
Some years ago, as a guide to the injection of proper amounts of bacteria and their toxins for the production of immune substances the weights of the injected rabbits were recorded. It soon became obvious that animals receiving doses larger than the ideal began to lose weight and the immune body production fell off in proportion to the weight loss. Since that time it has been our custom to record the weights of all stock and experimental rabbits twice a week and, if possible, to use no animals that have not been weighed at least twice. Without detailing all of the data that have been gathered it is possible to make a few generalizations.

1. Animals acquired from dealers show a rapid weight increase (200-500 gm.) in their new environment. If such a gain does not occur, it is unwise to begin any lengthy experiments until the animal does show a gain. In most instances when the animal does not show a weight increase within seven to ten days it dies within two to three weeks from either obvious or unknown causes.

2. Under the minor variations of cage environment, the healthy rabbit rarely loses more than 200 gm. in weight.

In Fig. 5 the weight changes of a pair of normal and a pair of experimental animals are recorded. U5A-1 and U5A-4 are normal brother and sister and the records shown are similar and almost identical with the records of the other four in this litter. It will be seen that the growth curve for the first year is practically a straight line. It is interesting that slight weight losses occurred when the animals were mated. The degree of weight loss is comparable to that occurring when rabbits are starved for the eighteen hours preceding a bleeding. The most obvious suggestion is that during the mating period there is no time for food.

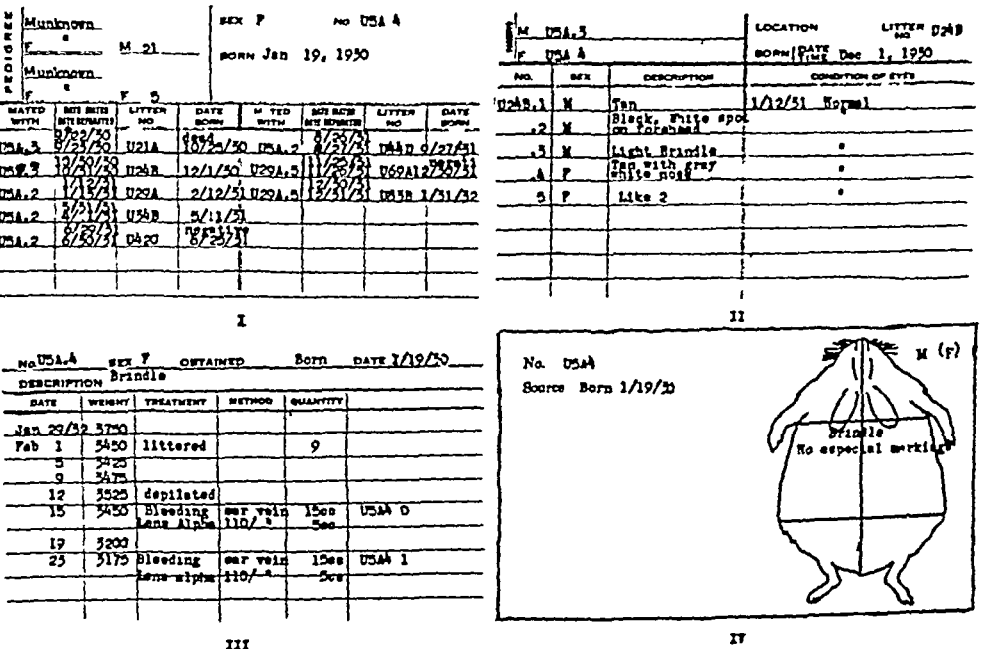
Following each successful mating there is a significant weight gain of 350 to 500 gm. in the female. This rise is apparent within two weeks after the mating and is the best indicator of the pregnancy. In passing it may be mentioned that the serum of a two weeks pregnant animal is very turbid and fatty even after a preliminary starvation. These signs indicate a pregnancy, but the young may



5. 2. 1. 1.

not be viable. If the young are dead the animal will either abort with a rapid fall in weight or retain the dead fetuses. In the latter case, there will be a slowly falling or stationary weight curve while the dead fetuses are converted into lithopedions or are slowly absorbed.

Following the birth of the young there is a sharp weight loss which is about equal to the previous gain. The normal animal holds this weight and balances its increased metabolism. Following the March, 1931 pregnancy of U5A 4 there was a marked loss in weight. On examination it was found the animal had a large tumor that involved two breasts. This broke down and drained in a few days. Accompanying the drainage and healing the previous weight loss was rapidly regained.



No U5A 4

SEX F

OBTAINED

BORN DATE 1/19/30

DESCRIPTION Brindle

DATE	WEIGHT	TREATMENT	METHOD	QUANTITY
Jan 29/32	3750			
Feb 1	5450	littered		9
3	5425			
9	5475			
12	3525	depilated		
15	5450	Bleeding	ear vein	15cc U5A 0
		lens alpha	110/4	5cc
17	3200			
23	5175	Bleeding	ear vein	15cc U5A 1
		lens alpha	110/4	5cc

No U5A 4

Source Born 1/19/30



M (F)

With the beginning of warm weather, U5A 4 while nursing a litter lost considerable weight. This, it is believed, was not due to the nursing burden but was directly associated with the temperature rise as a considerable number of rabbits, male and female, showed a similar loss.

U5A 4 did not litter during the summer months. In this it follows the general rule. During that summer, although numerous animals were mated no pregnancies resulted. Like U5A 4 they all had records of previous litters and all littered again during the Autumn.

The weight curve of the male U5A 1 parallels the female except for the pregnancy changes and the summer weight loss. In the Autumn of 1931 it was noticed that his head was beginning to rotate in the manner characteristic of middle ear infection. Shortly after this he developed a definite nasal discharge with a moderate amount of sneezing. From this time he showed a constant

Fig 6

weight loss and at autopsy a complete consolidation of the lungs was found. The weight loss seemed associated primarily with starvation as the slightest stimulus, auto or external, brought on the barrel rolling associated with torticollis, and he was unable to obtain an adequate amount of food.

The records of rabbits 29 and 132 show the weight changes associated with experimental procedures that may or may not be fatal to the rabbit. These weight changes seem largely dependent upon the general reaction of a rabbit to infection. A sick rabbit is drowsy and lethargic and takes practically no food or water, while a healthy rabbit eats continually. Correlated with this, apart from any destruction of tissue by the toxic substance, the animal with a chronic infection shows a marked loss of abdominal fat at autopsy. For example, rabbit 132, dead two weeks after the injection of staphylococcus toxin showed a complete loss of abdominal fat with no other demonstrable lesions. This toxin is mainly a heart and respiratory poison and after its injection the animal responds with a slowed respiration, marked diarrhea, and a general stuporous state, during which time little or no food is taken. In general, rabbits experimentally infected with organisms or substances lethal for the rabbit show a weight loss that varies with the severity of the induced condition. From the weight curve alone, it is not impossible to predict the death of the rabbit within a few days.

#### SUMMARY

Methods for the care and breeding of rabbits in the laboratory have been outlined. A detailed record has been presented of the weight changes occurring in normal animals and in a pair of animals injected with substances lethal to the rabbit. From the results, and other data not detailed, it is concluded that a weight record is an excellent guide to the clinical condition of the rabbit.

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# HINTON TEST FOR SYPHILIS

## THIRD MODIFICATION<sup>c</sup>

WILLIAM A HINTON, M D , BOSTON, MASSACHUSETTS

IN the management of syphilis, a growing reliance on serum tests demands continuous research to improve them. As a consequence of research so directed it became needful to make a third modification of the Hinton test for syphilis<sup>1 2 3</sup>. In its modified form as presented here, the accuracy of the test has been increased, the technique has been simplified and the interpretation is based on a set-up of one tube instead of four tubes for each serum. Though simplified, it requires precision in execution because as is true of any sensitive method, consistent and accurate results cannot be obtained if minor variations in technique are allowed to creep into the routine.

### EQUIPMENT

The equipment required for the test is as follows:

1 Test-tube racks holding twenty to one hundred and sixty tubes. To simplify the numbering and pipetting of the serums, these racks should be constructed to hold ten or twenty tubes in a row.

2 Serum tubes 10 mm inside diameter and 100 mm long. The diameter of the tubes should be approximately uniform.

3 A water-bath for heating serums, kept at 55° to 56° C.

4 A Wassermann bath preferably, or an incubator, kept at 37° C. The water in the bath should be changed frequently to prevent a deposit from forming on the outside of the tubes. The Wassermann bath is preferred because by its use the test is from 3 to 5 per cent more sensitive.

5 A centrifuge with which a speed of 2000 revolutions a minute can be obtained.

6 A thermometer that registers maximum and minimum temperatures.

7 Graduated 100 c c and 250-c c cylinders, for measuring the reagents.

8 Dropping pipettes with rubber bulbs, of about 75 c c capacity.

9 Serologic pipettes of 10 c c capacity, graduated in tenths for measuring the serums, and 5-c c or 10 c c serologic pipettes for measuring reagents and adding them to the serums.

10 Thick-walled Erlenmeyer flasks<sup>4</sup> of 125 to 500 c c capacity, with an inverted V-shaped partition blown in the bottom, for mixing glycerinated indicator (Fig 1). This partition should produce two semicircular compartments, each of which should hold from 3 to 5 c c in flasks with a capacity of 125 or 250 c c, and from 8 to 10 c c in flasks of 500 c c capacity. Flasks of this type are not listed in any of the catalogues. I have had mine especially modified for this purpose by Macalaster Bicknell Company of Cambridge, Massachusetts.

<sup>c</sup>From the Department of Clinical Research of The Boston Dispensary.

## PREPARATION OF REAGENTS

The reagents used in the test are (1) cholesterinized heart extract, (2) 5 per cent salt solution, and (3) 50 per cent glycerol solution

1 Cholesterinized heart extract This is prepared as follows Dried ground beef heart muscle (Bacto-Beef Heart, Dehydrated, Digestive Ferments Company) is extracted by putting 100 gm \* of the powder and 400 cc of ether (anesthesia) in a wide-mouthed glass-stoppered bottle and shaking thoroughly by hand for ten minutes The solid material is allowed to settle for five to ten minutes, and then as much of the ether as possible is poured through filter paper The ether is discarded The extracted tissue is scraped from the

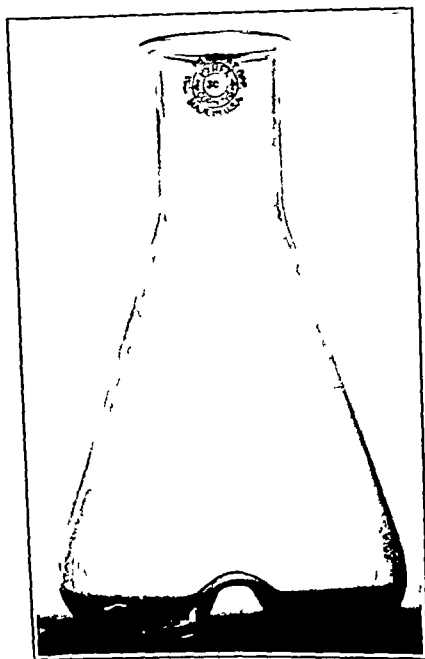


Fig 1—Inverted V-shaped partition blown in the bottom of an Erlenmeyer flask.

filter paper and replaced in the bottle for further extractions A total of five separate extractions must be made with 400 cc of fresh ether for each extraction The main portion of extracted tissue should not be allowed to dry between extractions A new filter paper should be used for each filtration After the final extraction the extracted tissue is dried on the filter paper This dried residue, which contains the ether-insoluble constituents is weighed it is then extracted for three days at room temperature ( $17^{\circ}$  to  $20^{\circ}$  C) in a glass-stoppered bottle with 95 per cent ethyl alcohol, in the proportion of 5 cc of the alcohol to each gram of residue The contents of the bottle are shaken vigorously by hand for five minutes three times each day The tissue is removed by filtering into a graduated cylinder, the alcoholic extract is measured and transferred to a glass stoppered bottle and cholesterol is added to the

\*The extraction of larger or smaller amounts of the powder in one operation has appeared to yield an inferior reagent

extract in the proportion of 0.4 gm to each 100 cc. The cholesterol is best dissolved by warming, at  $37^{\circ}\text{C}$ , in an incubator or water-bath, with occasional shaking. (It should not be stored in a refrigerator, for chilling will precipitate the cholesterol, if, by inadvertent chilling, the cholesterol should precipitate, it must be redissolved before use.)

Such cholesterolized heart extracts have been kept in our laboratories in colorless glass stoppered bottles, at room temperature for a period of more than two years.

Cholesterol obtained from Merck and from the Digestive Ferments Company has given identical results when compared. All of the many cholesterolized heart extracts prepared in accordance with these directions have given almost precisely the same results.

2 A 5 per cent solution of sodium chloride (C P) in sterile\* distilled water to each 4500 cc of which 10 gm of salicylic acid (C P) should be added. In the paper a solution so prepared will be referred to as 5 per cent salt solution.

The salicylic acid not only helps to preserve the potency of glycerinated indicator (described later), but also minimizes slight changes which occur with some negative serums.

3 A 50 per cent solution of glycerol prepared by mixing equal volumes of glycerol (C P) and sterile distilled water.

The 5 per cent salt solution and the 50 per cent glycerol solution keep indefinitely.

#### TECHNIC OF THE TEST

1 Centrifuge the blood if necessary, and with a long dropping pipette remove each serum from its clot and deliver into an appropriately labelled serum tube. To avoid contamination of one serum with another, after each serum specimen has been drawn off the dropping pipette should be thoroughly rinsed at least three times with sterilized physiologic salt solution (each time discarding the rinsing solution), and to minimize bacterial contamination, after every twenty serum specimens have been drawn off the washing bottle (about 200 cc capacity) should be emptied and filled with fresh salt solution.

2 Heat the serums in the inactivating bath at  $55^{\circ}\text{C}$  for thirty minutes. Be sure that the level of the water in the bath is above every serum specimen.

Extreme care should be taken to keep the water at  $55^{\circ}\text{C}$  throughout the entire period of inactivation, as erroneous results may be obtained when the temperature falls even one or two degrees below this point. Inactivation at a temperature above  $56^{\circ}\text{C}$  decreases the strength of the reactions.

Serums should be inactivated just before testing, as those which may give strongly positive reactions when tested a few hours after removal from the clot occasionally give negative or weak reactions when kept for even as short a time as a day.

Should it be necessary to retest a specimen, always use serum freshly separated from the clot, rather than that which has been inactivated twenty-four hours previously.

3 Set up the racks with one properly numbered serum tube for each specimen to be tested. Be sure that the tubes are clear and clean.

To clean the tubes, rinse them thoroughly, as soon after use as possible, with tap water, and then fill each one with a warm solution of 5 gm of sodium hydroxide in 1000 cc of tap water, allow the tubes to remain immersed in the solution for about two hours,

\*No attempt need be made, however, to keep materials or glassware strictly sterile. The purpose is simply to avoid needless gross bacterial contamination which seriously interferes with the accuracy of the test.

and wash *thoroughly* with hot water to remove the alkali. This process usually completely removes, without the use of a test tube brush, any deposit which may have remained from previous use (Boiling in this caustic solution corrodes the tubes). Sterilize in a hot air oven. This will minimize the possibility of bacterial growth, which may occur in the prolonged incubation. Other glassware should be cleaned in essentially the same manner.

4 With a 1-c c pipette graduated in tenths, measure 0.5 c c of each heated serum into a separate tube, that has been properly labelled. (Use a separate pipette for each serum.)

It is well to use several strongly positive and several negative serums as controls, particularly when only a few tests are to be made at a time.

5 Compare the appearance of each of the pipetted serums with the appearance of the serum in the tube from which it was taken, in order to be certain that there has been no error in pipetting, or in labelling the tubes.

6 Prepare glycerinated indicator as follows (adhering strictly to the directions for mixing, because incorrect mixing causes unreliable reactions). Pipette one part of the cholesterinized heart extract into one compartment of the Erlenmeyer flask with the inverted V-shaped partition in its bottom, and 0.8 part of the 5 per cent salt solution into the other side.

Great care should be used to avoid admixture of the two solutions when the salt solution is pipetted into the flask. A 125 c c flask is suitable for the preparation of 30 c c of glycerinated indicator, a 250 c c flask for 60 or 90 c c of glycerinated indicator, and a 500 c c flask is to be preferred for the preparation of larger quantities.

Mix the cholesterinized heart extract and salt solution by shaking the flask rapidly from side to side for one minute. Let the mixture stand for at least five minutes—this is important. Add 13.2 parts of the 5 per cent salt solution and shake thoroughly to obtain complete mixing. Finally, add fifteen parts of the 50 per cent glycerol solution and shake until the suspension is homogeneous. Thirty cubic centimeters of the glycerinated indicator is the smallest amount which can be mixed satisfactorily at one time, and amounts as great as 210 c c can be prepared with equal success.

As glycerinated indicator will remain unimpaired in strength for one week if kept in a refrigerator at a temperature of 8° C or lower, enough may be mixed at a time for a week's use.

7 With a clean 10 c c pipette, add, not more than thirty minutes before incubation, 0.5 c c of the glycerinated indicator just described to each serum.

8 Pipette 0.5 c c of the same indicator and 0.5 c c of the 5 per cent salt solution into an empty serum tube. This serves as a control, the purpose of which will be explained later.

9 Incline the rack to an angle of about 45°, and then shake by thrusting it quickly upward and forward, then downward and backward, with sufficient speed to cause the fluid to travel halfway up the tubes. At least three minutes of this motion are required for accurate results. The presence of small bubbles at the surface of the fluid in many of the tubes is the only safe criterion of adequate mixing of the serum with the indicator. This is important.

10 Select the tubes which contain serums that show hemolysis, or cloudiness due to bacterial growth, and test according to the quick method to be described later in the paper.



11 Place the rack of tubes in the Wassermann bath or incubator at  $37^{\circ}\text{C}$  and let it remain in the Wassermann bath for sixteen hours (conveniently from 5 P.M. to 9 A.M.), or in the incubator for eighteen hours. Do not disturb the contents of the tubes before reading.

12 At the time of removing the tests from the bath or incubator record the temperature readings shown by the bath or incubator thermometer, and note the readings on the maximum and minimum thermometer. For dependable results the temperature should not fall below  $34^{\circ}\text{C}$  nor rise above  $39^{\circ}\text{C}$ .

#### READING THE TESTS

To read the tests, sit in front of a window, but not in the direct sunlight (The light must be good, and for this reason good artificial light must be provided for reading on dark days or at night). Lift each tube carefully from the rack, and holding it at the level of the eye look at it in the direction of a darkened background on either side of a window or of a suitably placed light. Look for any clearing of the fluid and for a significant\* ring or band at the top of the meniscus of the fluid. If such a ring is not seen with the tube in this position, incline it to an angle of  $45^{\circ}$  and observe further. At the same angle, gently rotate the tube by rolling it between the fingers and again look for a ring. Finally, the tube is shaken to detect even the faintest precipitate.

#### INTERPRETATION OF TESTS

The reactions are designated as positive, negative, doubtful, and unsatisfactory, without indicating the intensity of the change in positive reactions.

The simplicity of this method of recording and reporting has the advantage of not confusing physicians by implying that the test is in any way a quantitative reaction. After a careful study, I find that, as in the Wassermann test, the intensity of the reaction of this test bears no relation to the clinical condition of the patient.

*Positive Reactions (recorded +)*—A ring or band of coarse granules or flakes of lipoids, approximately 0.2 mm to 1.5 mm wide, slightly to moderately, but not strongly adherent to the walls of the tube is present at or a little above the level of the meniscus. This ring or band is accompanied by complete clearing of the fluid, except for large agglutinated masses of lipoids or by partial clearing with only slight agglutination. Gentle shaking causes the ring or band to disperse and the particles to become visible as a definite precipitate throughout the fluid. In rare instances there is no ring, but a fine precipitate becomes visible on shaking. A positive reaction, if definite, is valid even though the serum shows evidence of bacterial contamination or hemolysis.

*Negative Reactions (recorded -)*—There is no clearing, no ring, no band, no precipitate—in fact, there is no visible change.

*Unsatisfactory Reactions (recorded "Unsat")*—The reaction is designated as unsatisfactory (except when it is definitely positive) whenever the blood or its serum has partially decomposed. This decomposition visibly manifests itself by hemolysis, and by cloudiness due to bacterial growth. In the test, hemolyzed serums frequently produce a whitish ring, which differs from the

\*To be significant, a ring must be only slightly adherent to the wall of the tube and must possess coarse granules or fine flakes which have a tendency to creep up the wall of the tube in a thin film when the tube is gently shaken.

significant ring found in positive reactions in that it sticks to the tube even after vigorous shaking. In the event of bacterial contamination, there is a filmy white aggregation of lipoids at the top of the fluid, which on first observation may be confused with a positive reaction. With only a little experience, however, one can readily distinguish between the coarse granules or the flakes of a positive reaction and the fine filmy cloud due to contaminated serum. If a serum shows hemolysis (as manifested by redness greater than that produced by a solution of 0.1 c.c. of blood in 3 c.c. of distilled water), or if it shows evidence of bacterial contamination at the time of reading, the reaction is designated as unsatisfactory. This interpretation should be made because even the moderately hemolyzed or bacterially contaminated serum of a known syphilitic will very often give a negative reaction, and because, as will be shown later, a negative reaction with a proper serum has great accuracy in excluding syphilis.

*Doubtful Reactions (recorded  $\pm$ )*—If only a slight granularity is seen on shaking, beyond that observed in the control tube (containing 0.5 c.c. of indicator and 0.5 c.c. of the 5 per cent salt solution), or if only a slightly flaky or slightly granular ring is seen, the interpretation must be guarded. Centrifuge for ten minutes, at high speed (about 2000 revolutions a minute) the tubes which show such reactions. Also centrifuge all tubes having serums that are somewhat opaque in themselves, but are not opaque because of bacterial contamination or hemolysis, since opacity may mask a doubtful reaction. It is important to restrict the use of the centrifuge to this condition and to the quick method to be described, otherwise, doubtful reactions will very frequently occur with the serums of nonsyphilitic persons. Centrifuging, as indicated by Eagle<sup>5</sup> causes the particles of an indefinite precipitate to cohere so as to be easily visible. The reaction is considered doubtful if at the meniscus a thin film of lipoids is formed which, on shaking, breaks up into fine flakes or coarse granules that persist as a precipitate even after vigorous shaking. When centrifuging does not markedly intensify the precipitate, the reaction is called negative.

#### A QUICK METHOD THAT DETECTS MOST OF THE POSITIVE REACTIONS

The quick method to be described is advantageous in two types of cases.

1. Whenever there is immediate need for a report the routine procedure should be carried out up to the point of incubation, but before incubation the tests in question should be centrifuged at high speed (about 2000 revolutions a minute) for ten minutes. Immediately after centrifuging, the readings should be made. Plainly visible clearing and a well-marked precipitate, seen on shaking, denote a positive reaction. On the other hand, the presence of only a very fine precipitate visible on shaking indicates no more than a probable positive reaction. The absence of a precipitate is reasonable but not conclusive evidence of a negative reaction. The tubes that show a probable positive reaction or a negative reaction should be well shaken and then placed in the water-bath for sixteen hours or in the incubator for eighteen hours, after which the reading in routine manner.

2 Specimens that are hemolyzed or bacterially contaminated should be tested by the quick method to avoid their further deterioration. In reporting the results of such tests it is important to know that, in proportion to the degree of deterioration, either hemolysis or bacterial contamination tends to prevent a positive Hinton reaction with serum from a syphilitic person. Therefore, tests that show a positive reaction with such serums should be so reported, but all others should be reported as unsatisfactory, without further incubation.

#### SIGNIFICANCE OF THE TEST

A positive Hinton reaction almost always indicates syphilis. Nevertheless, a single positive reaction must not be the basis for a diagnosis of syphilis unless supported by definite clinical evidence of the disease. Whenever a single positive reaction is unaccompanied by definite clinical signs of syphilis checks should be made by tests on at least two additional specimens of blood before concluding that the positive reaction is dependable. If the test is used in this way practically no incorrect diagnoses of syphilis will be made. I have noticed particularly that neither rheumatic fever nor pneumonia appears to cause with the Hinton test false positive reactions, as are so frequently encountered with the Wassermann test. As a guide during treatment, a positive reaction usually indicates that continuation of the treatment is necessary to prevent relapse. In cases where prolonged intensive treatment has not caused the positive reaction to disappear, however, there is a question as to the significance of this unyielding ("persistent") positive Hinton reaction. I do not feel that the test has been in use long enough to offer an answer to this question. I do believe, however, that continuous clinical observation is necessary in all such cases, and that most of them should have some type of antisymphilitic treatment as well. My own experience, as well as that of others,<sup>6</sup> shows that during intensive treatment positive Hinton reactions almost always persist a third longer than positive Wassermann reactions, and in many instances more than twice as long. Inasmuch as negative Wassermann reactions often occur prematurely during treatment as demonstrated by frequent serologic or clinical relapses, or both, the persistence of a positive Hinton reaction would indicate a superiority of the Hinton test as an aid in the management of syphilis, especially in cases where modern treatment is begun during the first weeks or months of the disease.

A doubtful Hinton reaction suggests syphilis, and occurs for the most part in patients in the beginning of the primary stage, or in syphilitics who serologically, have responded almost completely to treatment. In my experience the doubtful reaction has occurred in from 5 to 15 per cent of persons in whom syphilis was excluded with reasonable certainty.

A negative Hinton reaction is seldom erroneous, except during the first few days of the primary stage. Nevertheless, a single negative Hinton reaction alone is insufficient reason for excluding syphilis. In a study of 161 cases of known syphilis of three years' or less duration, in which during treatment, 929 Hinton tests (counting all, from the first positive to the last positive, inclusive) were made, there occurred but 6 per cent (54) false negative reactions. In this same group, three or more consecutive negative reactions followed by positives occurred in only 3 per cent (5) of the cases. Thus, even three negative

reactions are not necessarily decisive evidence of cure of a syphilitic person, though such a finding seems to be the best available evidence of permanent relief, aside from continued clinical observation. A basis for this belief arises from the fact that of the patients studied, 90 per cent of those who had three or more consecutive negative reactions had shown neither clinical nor serologic signs of relapse.

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A PRACTICAL TECHNIC FOR BLOOD CULTURES\*

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THE demands for varied bacteriologic technic in a large general hospital render the simplest procedures the most expedient. Blood culture technic in particular would seem to require the least possible handling of inoculum so as to allow for minimal chance of contamination and maximum accuracy of findings. The following procedure has been found to give consistent results particularly for cases of endocarditis and pneumonia.

*Technic*—About 20 c c of blood is withdrawn from the median basilic vein under sterile precautions and added to 10 c c of citrated saline (2 per cent sodium citrate in 0.85 per cent saline). The tube of blood is allowed to stand until a good portion of the red blood cells have settled. Pipette about 5 c c of the settled blood cells and transfer equal portions to two tubes of melted agar (temperature 45° C). Mix well and pour plates. The second plate will receive the heaviest concentration of red blood cells and the most bacteria. Transfer 15 to 20 c c of hormone broth, reaction 6.8 to 7.2, to the test tube in which the blood was collected. This tube will contain the major portion of the plasma and the residue of blood cells. Large potato tubes containing 15 to 20 c c of hormone broth are prepared. The final broth culture usually approximates 35 c c in volume. Incubate at 33° to 36°.

The plates and tubes are observed daily for the first three or four days, but are not opened unless positive culture is indicated. On the third or fourth day a sample of culture is removed from the depth of the broth and centrifugalized preparatory to the examination by Gram's technic. A transfer for verification by subculture is generally made at this time. Samples from the sediment are

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examined in like manner on the seventh, fourteenth, and twenty-first days and negative cultures are not discarded until the third or fourth week. The plates may be discarded at the end of the first week.

Certain theoretical considerations which have been borne out by findings have influenced the employment of the above procedure. Of prime importance is the dilution of bactericidal content of the blood serum by the sodium citrate solution. Next in importance is the fact that nonmotile bacteria may be held to the blood cells as colloidal particles and would settle accordingly. It would be expected that delicate organisms, few in number, might be protected by the sediment of blood cells during the period of lag.

*Gross Findings*—In many instances where plates have been negative, organisms have been found in profuse numbers in the blood cell sediment. Pneumococci and enterococci have been found to cloud supernatant broth faintly and the former can be typed by means of the precipitation method of Krumwiede and Valentine. Enterococci may be identified by means of positive aesculin fermentation and negative methyl red test.

Meningococci and gonococci grow profusely in the depth of the red blood cells, growth starting upon the individual cells and continuing as a gelatinous semiopaque ring at the meniscus of the broth after the bottom layers of culture have been disturbed. Meningococci are more resistant to heat when protected by the sediment of red blood cells.

*Micrococcus albus*, when present even in small numbers, grows in the layer of white blood cells at the bottom of the tube and continues to grow as a slimy white ring with trailing masses at the meniscus of the broth after the substratum of blood cells has been disturbed. Staphylococci generally cloud the supernatant with discrete accumulations of growth and produce a granular pellicle. Diphtheroid bacilli grow in a fine white layer above the layer of white blood cells in the substratum. Cases of septicemia approaching a fatal termination give equally profuse growth of organisms in the supernatant and substratum of broth cultures as well as in the plates. A profuse growth in the broth cultures from cases of epidemic pneumonia does not necessarily indicate unfavorable prognosis. Higher bacteria grow as round, fluffy, mold-like masses about the size of a pea in the layer of white blood cells or adherent to the wall of the tube. In blood agar plates colonies of them appear morphologically like *Streptococcus viridans*. Cases of bacterial endocarditis in which plates and supernatant broth appear grossly negative frequently yield profuse growth of organisms in the red blood cell substratum. Subcultures of the same on blood agar slants are so delicate as to seem negative.

*Conclusion*—A practical blood culture technic is described which provides for the isolation of delicate organisms in blood agar and broth by protecting them in a substratum of red blood cells.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M D, ABSTRACT EDITOR

**B C G VACCINE, Leukocytic Changes in Vaccinated Children, Dolgopoi, V B, and Statt, R H** Am Rev Tuberc 26 304, 1932

The blood picture of 37 children vaccinated with the Bacillus Calmette Guérin was examined at frequent intervals. The time of observation varied from one to thirteen months after vaccination.

The blood of 5 normal babies was examined, at similar intervals, for a period from four to seven weeks.

The fluctuations of the number of monocytes and lymphocytes that were observed in the vaccinated children were within the normal range of variations for the young children.

The fluctuations of the number of monocytes and lymphocytes were greatest in the newborn babies (the group of children vaccinated by the oral method), the older children (those vaccinated subcutaneously), showed the least changes in the blood picture.

An increase in the number of monocytes was often observed on the day of intracutaneous tuberculin test, or on the day following the test, irrespective of a positive or negative reaction to tuberculin. The increase in the number of monocytes after tuberculin tests was seen also in some children before the vaccination (probably a response to some nonspecific factor).

Nine cases vaccinated by parenteral routes showed a slight elevation of the number of monocytes between the first and the third day after vaccination.

A steady relatively high monocyte count and a low lymphocyte count, without any intercurrent infection, were observed in one case (oral vaccination), or in 27 per cent of all cases, after four months the blood picture of this child became normal. The child was well after two years of clinical observation.

A complete autopsy on one child that died of enteritis and pneumonia did not reveal any tuberculosis or tubercle-like lesions in the organs or in the lymph nodes, this child shows a high monocyte and lymphocyte count both before and after vaccination (oral route).

The slightest degree of hematologic changes after administration of the Bacillus Calmette Guérin, especially infrequency of a noticeable increase in the number of monocytes, indicates low tuberculogenic properties of this bacillus.

The hematologic response to the vaccination with the Bacillus Calmette Guérin, as expressed in the fluctuations of the number of monocytes and of lymphocytes, is not great. The blood picture cannot therefore, be used as an index of the process of immunization with the Bacillus Calmette Guérin, as proposed by Pittaluga and Garcia.

**SEDIMENTATION TEST, in Pulmonary Tuberculosis, Kaminsky, J, and Davidson, D** Am Rev Tuberc 26 282, 1932

The results of the red cell sedimentation test in a series of 500 cases are reported.

No definite relationship could be established between variations in the number of red cells and the sedimentation speed. (Red cell counts and sedimentation tests were done in a group of 92 cases and the number of red cells ranged from 4,150,000 to 5,990,000). A low color index was found to accompany a rapid sedimentation velocity.

In 48 normal individuals the sedimentation index ranged from  $1\frac{1}{4}$  to  $10\frac{1}{4}$ , with an average of 4.5 for the men, and from  $5\frac{1}{4}$  to  $11\frac{1}{4}$ , with an average of 9.15 for the women.

There is a close parallelism between activity of the lesion and the sedimentation rate in pulmonary tuberculosis. The group of patients, whose sedimentation graphs were hori-

zontal lines, showed the lowest incidence of positive sputa, cavity formation, pulse and temperature elevations and substandard weight. The group of patients whose sedimentation test graphs were vertical curves, showed the highest incidence of objective signs of activity.

There were no cases with active pulmonary tuberculosis showing normal sedimentation values on repeated tests. There was one instance in which a seemingly active case showed a normal sedimentation graph, but the graphs obtained in 5 subsequent sedimentation tests done during the following eight months were all abnormal.

In four patients who died at the institution, the sedimentation tests done within a month before death showed slower sinking velocities than the tests taken in the preceding months.

The sedimentation test is of considerable value as an indicator of activity. A single determination may be of some help in appraising a case of pulmonary tuberculosis and repeated tests may furnish much additional information as to the progress of the case and serve to estimate effectiveness of treatment.

#### **ARTHRITIS, Correlation of Experimental Streptococcic Arthritis in Rabbits With Chronic Rheumatoid Arthritis, Hadjopoulos, L G, and Burbank, R. J Bone and Joint Surg 14 471, 1932**

By incubating the whole joint the authors were able to demonstrate these infecting organisms in practically all lesions associated with chronic atrophic arthropathies. Streptococci were found in every part of the synovial tissues where pathologic changes could be detected. No streptococci could be demonstrated in compact bone, yet the spread of infection in bone tissue could be traced step by step through its medullary and haversian vascular supply. In the avascular cartilage the spread of streptococci was through infected bone tissue and synovial infiltration. Normal joints were subjected to the same procedure with uniformly negative findings.

To complete the picture of infection, the authors have recently studied the tendons and muscles in the immediate neighborhood of arthritic joints. We had no difficulty in demonstrating streptococci in tendons at the zone of nuclear proliferation. Muscles were extensively infiltrated with streptococci at their sheaths but in spite of great care the possibility of outside contamination in surface areas must be considered.

Throughout this study the progress of the disease could always be traced directly to the terminal blood vessels as is true in all infections of hematogenous origin. Despite the specific selectivity of the arthrotropic streptococci used in these experiments, internal organs, especially the liver and kidneys, were not exempt from pathologic changes of toxic nature involving their parenchyma. Nevertheless, streptococci could be demonstrated in the interlobular connective tissue of the liver and occasionally even in the hepatic vessels. This finding is highly significant in that it forcibly brings home the fact that chronic rheumatoid arthritis is fundamentally a systemic disease, and it explains the transient septicemias that occur intermittently in the course of the infection.

#### **MENINGOCOCCUS, Specific Precipitin Reaction Associated With Growth on Agar Plates, Petrie, G. Brit J Exper Path 13 280, 1932**

Characteristic haloes have been observed round colonies of "smooth" strains of meningococcus, pneumococcus and B dysenteriae (Shiga), when these bacteria were grown on agar plates which contained the homologous immune serum.

The haloes consist of a precipitate which is formed by the interaction of the specific polysaccharide with the homologous antibody.

A "rough" strain of pneumococcus, by virtue of the "rough" polysaccharide or the somatic nucleoprotein, forms haloes in the presence of a "rough" or a type specific serum, the haloes round "rough" colonies are extremely tenuous.

The haloes that are produced by the meningococcus and by both "smooth" and "rough" variants of the pneumococcus represent in the initial stage a uniform deposit of the precipitate, at a latter stage the precipitate tends to assume the form of Liesegang rings.

When the specific polysaccharide which has been isolated by Morgan from B dysenteriae

(Shiga) is deposited in suitable concentration upon an immune serum agar plate, the resulting precipitate takes the form of a typical halo with concentric zones

The ability to form specific haloes constitutes a test for distinguishing between the "rough" and "smooth" variants of bacteria

The technic follows

Agar plates were prepared as follows (1) With the addition of 2.5 per cent sodium chloride, (2) with 5 per cent normal horse serum, (3) the same as (2), but with 2.5 per cent sodium chloride, (4) with 5 per cent antimeningococcus serum (Type I), and (5) the same as (4), but with 2.5 per cent sodium chloride. The salt was added with the idea that it might intensify the "rough" appearance of colonies that were composed of salt sensitive cocci. The anti meningococcus serum was a mixture from four horses which were being immunized with Type I cultures, the mean agglutination titer was 1 in 450. Agar plates prepared in this way were inoculated with each of the two strains.

Three days later a delicate halo was seen round the colonies of the recent strain on the agar to which antimeningococcus serum had been added, but not round those of the old laboratory strain. The haloes were apparent on the plates, both with and without 2.5 per cent sodium chloride.

These observations, which are supported by further work, indicate that the halo represents a specific precipitate which is formed by the interaction of the antibody in the serum with the specific polysaccharide of the meningococcus, this is set free from the cocci in the colony by a process of secretion or autolysis, and diffuses into the agar round the colony.

#### **B TYPHOSUS, Value of Wilson and Blair's Bismuth Sulphite Medium for Isolation of, From Feces and Sewage Stewart, A. D., and Ghosal, S. C. Ind J M Res 20 341, 1932**

The authors found this medium much superior to McConkey's. The formula follows.

To 100 cc of melted 3 per cent agar are added 5 cc of a 20 per cent solution of glucose, 10 cc of a 40 per cent solution of sodium sulphite (anhydrous), and 5 cc of a standard bismuth solution. After boiling for two minutes an addition is made of 1 gm. exsiccated sodium phosphate dissolved in 10 cc of boiling water and 1 cc of 8 per cent solution of ferrous sulphate crystals. To it is then added 0.5 cc of a 1 per cent watery solution of brilliant green and 2 cc of absolute alcohol.

The standard liquor bismuthi is prepared by mixing 60 gm. of bismuth citrate with 50 cc of distilled water and then 20 cc of liquor ammoniac of specific gravity 0.880 and finally making the volume up to 500 cc with distilled water.

It has been stated by some that the blackness of the colonies depends upon the brand of bismuth citrate used, but different brands have given equally good results in the author's hands.

It should also be noted that the specific gravity of the liquor ammoniac as advocated by Wilson and Blair should be strictly 0.880, any deviation from this gives indifferent results.

#### **BLOOD Studies on Blood and Tissue Reactions, Harvey, W. F., and Hamilton, T. D. Edinburgh M J 39 285, 1932**

##### **I Notes on Technic**

This paper, which because of its detail does not lend itself well to abstraction, is referred to because of the many practical suggestions, based on experience, which it presents. Those interested in hemitologic methods will be well repaid by its perusal.

##### **II The Differential Blood Count**

The following conclusions are advanced.

There is not a very large error in making a single leucocyte total count provided that the necessary precautions are taken.

The differential cell count on a well made, and to a surprising extent on an ill made, film is significantly valid for diagnostic purposes.

There is evidence of long wave diurnal oscillation of leucocyte numbers and proportions but not sufficient evidence of a superimposed periodic short wave type of fluctuation.



All sorts of factors, such as sleep posture, heat and cold, muscular exercise, locality of examination, digestion and simple ingestion, may be regarded as traumatic and may produce leucocytic variation of great or less duration and may combine together to enhance or to mask the variation due to any one factor singly.

The authors emphasize the importance of a properly made smear of such a size that the entire area of it may be counted without undue labor.

### III The Peripheral Blood Picture Is the Image of the Focal Lesion

That the peritoneal fluid is closely related to blood fluid is a reasonable supposition. That it should, when debris, dead cells, desquamated cells and reparative cells or structures are excluded from consideration contain more or less the same cells as the blood is only what one would expect. This investigation therefore, in so far as it affirms a close correspondence in the relative proportions of these cells brings out the fact that the blood fluid in, and in the vicinity of, a disease focus has a similar cell composition and the same significance for the phase of the disease as has the peripheral circulating blood fluid. It is also contended that the peritoneal cavity is a lymph space or tissue space quite comparable with those of lesser dimensions elsewhere and that introduction of an irritant into the peritoneal cavity is to be compared with its introduction elsewhere in the body and to a traumatism. Again, it is only to be expected that, with a complex stimulus to cell exudate, such as may be supplied by a pathogenic organism continuing to multiply and perhaps distributed in several foci not all in the same stage of development, we should have a peripheral blood picture representing the superposition of a number of phases of focal reaction. This however does not negative the contention that the blood picture closely resembles, so far as its particular types of cells go, that of the focal disease from which it is concluded that the peripheral blood picture is the image of the focal lesion, whatever be the nature of the irritant.

### VACCINES, Clinical Reactions to, as Guides to Treatment, Thomas, W S, and Touart, M. D Am J M Sc 184 210, 1932

It is recognized, or should be, that the dose of bacterial vaccines is determined by the reaction produced in the patient and the authors propose, therefore, methods whereby the reactivity of the patient to the vaccine may be determined is a guide to their administration.

*Early Local (Wheal) Reaction*—The early positive reaction at the site of injection of a vaccine is a wheal at least 0.5 cm. larger in diameter than any which may appear at the site of the control injection. It frequently has an irregular outline or even pseudopods and is often surrounded by a zone of erythema (areola). Itching may be present. The wheal reaction is at its height in from 10 to 30 minutes and fades soon thereafter.

*Significance of the Early Local (Wheal) Reaction*—Occurrence of the early wheal reaction is taken to indicate that the vaccine causing it should be used in the treatment of the case. This reaction is of aid in determining subsequent dosage of the vaccine. Should the reaction be very marked, the treatment dose next following is not increased, other things being equal.

In order to record the wheal reaction the circumference of the wheal and of the areola are outlined upon the skin with pen and ink and numbered. When dry, the picture is transferred by contact to a sheet of damp white blotting paper and thus, when dry, is filed for record.

*Late Local Skin Reaction*—The late positive reaction at the site of inoculation is not unlike the Schick reaction in appearance, but in order to observe it properly its component features are noted by touch as well as by sight and with the assistance of the patient's sensations as described in response to questioning. The sum total of the symptoms are recorded graphically as they appear, change and disappear during the days following the test. Any or all of the following symptoms have been observed at the testing site:

(1) An indurated nodule, slightly or not at all elevated above the surrounding surface of the skin and about 0.5 cm. in diameter or larger, sometimes lasting for several or even for many days. Long endurance of it has come to be looked upon as a favorable prognostic sign if the vaccine treatment can be thoroughly carried out. (2) Redness of the skin over the nodule. (3) A surrounding zone of erythema (areola) from 1 to 10 or more centimeters in

average diameter (4) Tenderness on pressure, usually at the nodule only, but sometimes extending over the whole of an existing areola (5) Heat at the injection site or over the whole of the areola, usually beginning in from six to twelve hours and lasting for one or two days (6) Swelling of the whole areola (7) Lymphangitis, beginning in from eight to twelve hours and lasting for a day or two, has been observed in about 2 per cent of the patients who showed late local reactions (8) Pustule or vesicle in the nodule This is not due to infection, as repeated culturings have always proved sterile (9) Pigmentation of the surface of the nodule occurs late and is not often seen (10) Desquamation of the skin over the site of the disappeared nodule is not common but occurs so late that it has doubtless been overlooked at times Very rarely a slough has occurred at the site of the inoculation

The features of the late focal reaction may be recorded upon a graph in which N denotes a nodule 0.5 cm. in diameter, NN or N2 a nodule twice as large Redness of the nodule is denoted by the letter R, T indicates tenderness on pressure The findings of the late local reaction are read and recorded upon at least two successive days following the test A late reaction is considered to be positive when, in addition to any features occurring at the control site, there are present during 2 or 3 days, three or more of the component features named above Such reactions as vesicles and ecchymoses are considered to be positive even though occurring alone, and to indicate that the organisms causing them should be used in vaccine treatment of the case

*Significance of the Late Local (Skin) Reaction*—A positive late local reaction is taken to indicate that the autogenous vaccine causing it should be used in the treatment of the case A very marked late local reaction calls for a smaller amount of vaccine to be given in the next following therapeutic dose than does a slight reaction During the course of a series of treatment injections each late skin reaction helps the operator to determine the amount and the time of the next vaccine dose

*General Toxic Reaction*—This term has been selected to express the rather frequently seen symptom complex following vaccine injections and characterized by malaise, nausea, drowsiness, feverishness and occasionally swollen and tender lymph nodes Any or all of these symptoms may occur within from four to twelve hours after the administration of the vaccine This reaction occurs only in conjunction with a positive late local reaction

It signifies that an overdose of vaccine has been administered It does not appear to furnish evidence for or against a specific action of the vaccine causing it, but it is of aid in determining subsequent vaccine dosage

*Symptomatic Reaction*—This name is applied to the onset or increase of allergic symptoms such as bronchospasm, urticaria, joint pains, etc., following within forty eight hours after the administration of vaccine This reaction occurs only in conjunction with a positive local reaction

*Relief Reaction (Temporary)*—In 45 of the patients quick relief followed the testing or other injection of vaccines The relief obtained was either partial or complete and occurred within a few minutes or hours after the vaccine injection and lasted for hours or days or even longer

This reaction appears to indicate that a favorable dose of a specific desensitizing substance has been administered It is therefore of aid in fixing upon subsequent dosage

*Focal Reaction*—By this term it is intended to signify a lighting up of symptoms of infection at the site from which the vaccine organisms were obtained It appears, if at all, within from four to twelve hours after administration of the vaccine

A focal reaction is thought to indicate that the vaccine causing it is specifically related to a focus of infection but is not necessarily connected with the allergic or other symptoms that are under treatment It may indicate the need for focal surgery at a site hitherto unsuspected

*Recurrent Local Reaction*—This phenomenon consists of the reappearance of a faded late skin reaction, days or weeks after its disappearance It follows in from six to twenty four hours after the reinjection of a vaccine elsewhere in the body

At present this reaction does not appear to afford information that may aid in planning subsequent vaccine treatment

*Negative Reaction*—This term means, of course, the absence of local and constitutional effects from the injection of a vaccine

The absence of all reactions appears to indicate that the organisms used are harmless to their host and that they will be of no benefit in treatment and should not be so used

The plea is made for the adoption of certain standard procedures in all cases in which the employment of vaccine therapy is contemplated, namely

- 1 Careful selection of patients, so as to avoid attempting vaccine therapy in persons unfitted for the method

- 2 A complete bacteriologic survey of the patients and the preparation of standardized vaccines from all the organisms recovered, except those that are spore bearers

- 3 The performance of skin testing with all vaccines so obtained

- 4 Careful observation, recording and interpretation of the reactions that follow such tests

- 5 Application of the data thus obtained to the subsequent management of the cases

# REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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## Fungous Diseases

A COMPREHENSIVE discussion of molds responsible for disease and the pathologic changes consequent to their activity For further review notes see editorial in this issue

## Hautkrankheiten und Ernährung

URBACH'S volume on Diseases of the Skin and Nutrition With Special Reference to Dermatoses of Childhood, discusses the dietetic treatment of skin diseases Dermatologists in general are giving up the idea that most dermatologic lesions are purely local and are interpreting them in terms of general metabolism This volume represents a distinct forward step in this respect

The first or general portion discusses the influence of nutrition on the chemistry of the skin and on its biologic reactions Then follows a chapter on nutritional changes as causes for cutaneous manifestations This includes such conditions as the vitamin deficiencies, dehydration, general undernutrition, milk sickness, and the like Next follows a chapter on dermatologic lesions associated with alimentary tract infections and intoxications, one on dermatoses due to functional disturbance of the digestive organs The sixth chapter deals with dermatoses as causes for metabolic disturbances of internal organs and the seventh discusses food idiosyncrasy

Since Urbach, following the work that was inaugurated by Luthlen, has developed a special method of allergic therapy for the dermatoses, this section is of unusual interest

The author prepares peptones of the various foods commonly used, and feeds his allergic patient a specific peptone for each of the food articles which the patient is going to receive at the next meal In this manner he believes he accomplishes specific alimentary desensitization prior to the meal The method is described in detail and accompanied by the presentation of several illustrative cases

In the second section the specific dermatologic diseases are taken up alphabetically The dietary principles of therapy in each case are described in detail

## Lang's German-English Medical Dictionary

THIS volume, in its fourth edition, contains 56,500 definitions Only scientific and medical terms are included This is not a general German English Dictionary The common words of everyday usage may be found in the ordinary German English Dictionary This distinction between a general German English Dictionary and this Medical German English Dictionary corresponds to the distinction between Webster and Dorland in our own language

•Fungous Diseases A Clinical and Mycological Text By Harry P Jacobson M.D. Attending Dermatologist and Member of the Malignancy Board of the Los Angeles County General Hospital Thomas Springfield Ill 1932 pp 317 cloth  
•Hautkrankheiten und Ernährung mit Berücksichtigung der Dermatosen des Kindesalters Von Dr Erich Urbach Privatdozent für Dermatologie an der Universität Wien I Prof Dr W Kerl Mit 55 Abbildungen 8 Kurven und 10 Tabellen Cloth Pages 260  
Wien Verlag von Wilhelm Maudrich 1932  
†Lang's German-English Dictionary of Terms used in Medicine and the Allied Sciences with their pronunciation Revised and Edited by Milton K Meyers M.D. Neurologist to the Northern Liberties Hospital Chief of Serve Clinic St Agnes Hospital Consulting Neurologist to the Jewish Hospital Philadelphia etc Fourth Edition Enlarged Cloth Pages 926  
J B Lister & Son & Co Inc Philadelphia

Those who, with a hobbling acquaintance with German, have tried to dig out their own scientific translations will appreciate this volume. With its use the German compound words will not be quite as horrifying.

In the fourth edition, the editor has added a key to pronunciation for each word.

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### Medical and Surgical Year-Book

**D**URING the summer of 1929 the Physicians' Hospital of Plattsburgh undertook a period of undergraduate instruction for a limited number of medical students between their third and fourth years, major attention being given to cardiac disease. Lectures and clinics were given both by the local members of the Hospital and by invited guests from several of the medical centers. These papers were then collected and have been published in book form. In this form they become a very readable and very excellent symposium on cardiology.

The summer courses in cardionephritis for undergraduate students in medicine will be continued from year to year.

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### Clinical Interpretation of Laboratory Reports

**A** VERY comprehensive volume on the interpretation that may be made by the clinician of reports rendered by the laboratory. The information detailed is as brief as is consistent with the objective of the book. The volume will therefore have greatest appeal to the general practitioner rather than to the internist who would often wish to find more detail.

Some of the reports discussed will be especially helpful to the general men. Thus when a Wassermann report is returned as "hemolyzed" or "anticomplimentary," etc., the significance of this is explained.

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### The International Medical Annual†

**N**INETEEN THIRTY TWO marks half a century for The International Medical Annual. The 1931 volume is for the forty ninth year. This follows the general arrangement of previous volumes with subject matter arranged alphabetically throughout. This greatly facilitates ease in finding reviews of progress for the year. There is a cross index at the end of the volume covering sub topics.

While no review of progress in an annual ever makes any pretense of being altogether complete and inclusive, there is no doubt that few if any volumes are available which cover as thoroughly all phases of medicine.

The reviews are truly international in their scope but naturally there is a little pre dominance of British contributions. This should increase its value to Americans who may not have available all of the British medical literature.

In addition the editors provide an introductory review of outstanding advances that have been made during the year. In this way there is available an eight page review of the outstanding advances together with a five hundred and twelve page review of outstanding articles on the different subjects.

The illustrations are abundant and excellent.

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\*Medical and Surgical Year-Book. Physicians Hospital of Plattsburgh. Comprising Wednesday Afternoon Invitation Lecturers' Papers of the Cardiac Round Table. The First Beumont Lecture. Collected Papers by the Staff. Cloth. Pages 322. The William H. Miner Foundation. Plattsburgh, N. Y. 1930.

\*\*Clinical Interpretation of Laboratory Reports. By Albert S. Welch, A.B. M.D. Clinical Instructor in Medicine in the University of Kansas School of Medicine in Kansas City, Kansas. Director of the Laboratory of the Alfred Benjamin Dispensary and Attending Surgeon of St. Joseph's Hospital in Kansas City, Missouri. Formerly Pathologist to the Kansas City General Hospital and Chemist to the Coroner of Jackson County. With sixteen illustrations and a frontispiece in color. Cloth. Pages 366. P. Blakiston's Son & Company, Philadelphia, 1932.

†The International Medical Annual. A Year-Book of Treatment and Practitioners Index. Editors: Carey F. Coombs, M.D., F.R.C.P., and A. Rendle Short, M.D., B.S. B. Many Contributors. Forty-Ninth Year. Cloth. Pages 551. William Wood & Company, New York.

## Arterial Hypertension

THIS is a very readable review of our present understanding of hypertension into which the author has incorporated his own studies and a very adequate discussion of therapy in general and in specific

Dr Stieglitz emphasizes that hypertension is a disease of multiple etiology in which the causes may be roughly divided into those which initiate the progress of hypertension and those factors which tend to perpetuate the hypertension once it has been initiated. He groups the etiologic factors as infectious, intoxication (exogenous and endogenous) and hereditary. The hereditary factors consist in lessened resistance to fatigue of vascular structures, emotional temperament, and obesity.

The diagnosis of the etiology of one case of hypertension practically never resolves itself into a single etiologic factor. The author presents several illustrative cases bringing out this point. For example in one case, the etiologic factors were found to be (1) oral sepsis, (2) excesses in salt, (3) obesity, (4) heredity, (5) anemia, (6) nephritis. In another case the etiologic factors were (1) heredity (emotionalism), (2) pregnancy intoxication, (3) mild hyperthyroidism, (4) recent anxiety. In still another the etiology was (1) oral sepsis, (2) luetic infection, (3) plumbism, (4) alcoholism. Without an adequate appreciation that all of these factors are interacting either as initiating causes or perpetuating causes, the results of treatment will not be optimal.

The pathogenesis of hypertension is discussed in detail and very well illustrated graphically in a panel diagram which displays how irritation of arterial musculature produces muscular spasticity and increased muscle tone which in turn causes hypertrophy of the arterial muscle. Here we run into a small eddy of a vicious circle in which spasticity produces muscular fatigue which in turn results in muscular hyperirritability which produces more spasticity. The two latter factors, hypertrophy and fatigue, are responsible for muscular exhaustion and degeneration which gives place to fibrosis in the replacement of degenerated muscular fibers. This in turn results in the end state, arteriolar sclerosis.

As one might expect, a discussion of the treatment deals principally with the general supervision and only secondarily with drug therapy. The latter however is very adequately covered and a comprehensive presentation is given of the author's own experiences with the use of bismuth subnitrate as a slow but continuous vasodilator. A cursory reader might infer that he recommends the use of digitals in hypertension more generally than is his actual intention.

The monograph can be highly recommended.

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\*Arterial Hypertension. By Edward J. Stieglitz, M.S., M.D., Assistant Clinical Professor of Medicine, Rush Medical College, University of Chicago. Attending Internist, Chicago Lying-in Hospital. Assistant Attending Physician, Presbyterian Hospital. Foreword by Rollin T. Woodruff, M.D., Clinical Professor of Medicine, Rush Medical College, University of Chicago. Chairman, Department of Medicine, Attending Internist, Presbyterian Hospital. With 21 Illustrations. Cloth. Pages 280. Paul B. Hoeber, New York, 1930.

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Editor WARREN T VAUGHAN, M D

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## EDITORIAL

### Fungous Diseases

OLD acquaintances, looked upon as of little importance sometimes step into more prominent rôles. This appears to be the case at the present time with regard to the pathogenic fungi. Curiously enough fungi were recognized as causes of disease even before Pasteur laid the foundation of modern bacteriology. In 1677 Hooke found that the yellow spots which grow on rose leaves consisted of fungi whose presence he attributed to spontaneous generation. Langenbeck discovered the parasitic fungus responsible for thrush in 1839. In the same year Schoenlein pronounced the fungus origin of favus. The etiology of tinea circinata was announced in 1884, that of pityriasis versicolor in 1846. For years therefore, dermatologists have been interested in the activities of parasitic molds in the skin and on the mucous membranes. Occasionally members of this group of plant life have been known or suspected as causing internal disturbances such

as actinomycosis, sprue, etc. Recent studies in these and other fields have however given greater prominence to the molds as possible etiologic agents of disease.

Molds are members of the vegetable kingdom, more highly developed than bacteria, but differing from the higher plants in that they do not become differentiated into roots, stems and leaves. They differ from the algae in that in the absence of chlorophyll they cannot manufacture their food from the carbon dioxide of the air but must live off of previously manufactured organic matter. Like bacteria, fungi may be parasitic, saprophytic or facultative parasitic. There are a great number of varieties and species.

Jacobson, whose primary interest is from the dermatologic viewpoint and who has recently published a monograph on *Fungous Diseases* classifies these as (a) primary cutaneous mycoses with usually no definite systemic involvement, (b) primary cutaneous or mucous membrane infections, or both, with frequent systemic involvement, and (c) primary systemic infections with occasional instances of skin or mucous membrane involvement.

In the first group, those limited to the skin, there are two subdivisions, which he designates saprophytic and parasitic. The former is essentially an invasion of the upper layers of the cutis, never becoming deep and is represented by such common manifestations as tinea versicolor and erythrasma. The second or parasitic group penetrate more deeply but here again there is usually no systemic involvement. This group is represented by the tinea infections of the hairy regions and by the familiar trichophyton group.

The second major classification, with skin or mucous membrane involvement and frequent systemic activity include monilia infections, more commonly known as the infections due to yeast-like organisms and the group of devastating but fortunately infrequent dermatologic or systemic diseases which include madura foot, sporotrichosis, blastomycosis, actinomycosis and coccidioidal granuloma.

Jacobson's third classification, systemic infections with occasional instances of skin or mucous membrane involvement is represented by two types of mold infections, torulosis and aspergillosis. The former shows some predilection toward central nervous system involvement, also pulmonary involvement, while the latter involves the lungs by preference.

Although certain of the cutaneous manifestations of these various mold infections are characteristic, enabling one to reach a diagnosis from inspection only, others require biopsy for accurate etiologic diagnosis. When internal organs are involved (bones, lungs, central nervous system, parenchymatous tissues), the clinical picture as a rule does not suggest itself as that of a mold infection but resembles more or less closely some other type of pathology, particularly pyogenic infection. Undoubtedly the apparent rarity of many of these diseases is due merely to the fact that, not being suspected laboratory studies have not been made with this in mind. It would be well when examining material from apparently infectious sources within the body, to always bear in mind the possibility of fungus infection. The constantly increasing number of cases that have been treated often for years as pulmonary tuberculosis which are found eventually on sputum examination to be due to mold infection and which often respond beautifully to administration of iodides bears this out.



The wide distribution of molds in nature, in dust, in the air we breathe, and the convincing evidence presented in the last few years that one may become specifically allergic to molds also suggests the occasional importance of fungi in the etiology of disease

## REFERENCE

Fungous Diseases. A Clinical and Mycological Text. Harry P. Jacobson, Attending Dermatologist and Member of the Malignancy Board of the Los Angeles County General Hospital, Thomas, Springfield, Ill., 1932, pp. 317

W T V

# *The Journal of Laboratory and Clinical Medicine*

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## *CLINICAL AND EXPERIMENTAL*

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### THE CLASSIFICATION OF ALLERGIC DISEASES\*

WITH REFERENCE TO DIAGNOSIS AND TREATMENT

ARTHUR F. COCA, M.D., PEARL RIVER, N. Y.

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**D**URING the past twenty years we have witnessed the rapid growth of a new medical specialty which is based upon a newly recognized principle of the causation of disease. This principle lies in the surprising paradox that antibodies, which had previously been known to medicine chiefly as protectors against disease, often represent the actual cause of disease.

This concept was formulated on the basis of animal experiments in anaphylaxis long before the peculiar antibodies of the corresponding allergic conditions in the human being were discovered.

These peculiar human antibodies are not demonstrable in all of the allergies, not in any of those exhibited toward the alkaloids such as quinine, chemicals, such as nickel and arsenic and the excitants of what was formerly referred to as dermatitis venenata, but is now known as contact dermatitis. They are also often missing in cases of urticaria or angioneurotic edema especially those in which there is no associated involvement of the mucous membranes (bronchial, nasal, gastrointestinal). They are however, regularly present in hay fever, in bronchial asthma when the asthma is caused by a specific antigenic excitant, in the inherited eczema, which is associated by heredity with hay fever and asthma, in gastrointestinal allergy and possibly in some other clinical expressions of allergy. They have been given the name "atopic reagins" or skin sensitizing antibodies.

When the reagin containing serum of a hypersensitive person is injected into the skin of a normal person, the injected site soon becomes sensitive to the same substances to which the patient is sensitive and this passive skin sensitivity persists for several weeks.

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\*Read before the Montefiore Hospital Medical Staff, Pittsburgh, Pa., November 24, 1931.  
Received for publication, December 12, 1931.

Evidently if the allergic person's serum is capable of sensitizing another person's skin his own skin must be constantly sensitized by the blood passing through it, consequently, if the sensitive person's skin fails to react to a certain material and if the test was properly applied, then it would seem to be useless to look for reagins in his blood with the method of passive skin sensitization which I have just described.

Practically, however, this is not the case, since Walzer finds that in some instances, in which the reagin content of the patient's serum is so low that there is only a slight though significant difference between the reactions in the passively sensitized site and the control (see below under Indirect Test), the reactions in the patient's skin are too slight to be recognized as specific.

For all allergic conditions in which reagins are participating, there are two kinds of tests which are used in searching for a specific excitant, these are the cutaneous tests and the ophthalmic test.

### CUTANEOUS TESTS

It seems probable that the controversy over the relative merits of the scratch method and the intracutaneous method of applying the skin test will end in a draw, because the two methods have been found to yield the same results provided they are properly applied with suitable extracts by competent observers.

The quantitative comparative tests of Fineman<sup>1</sup> showed that if sufficiently strong fluid extracts are used with the scratch technique, the results are the same as those obtained with the intracutaneous method with the more dilute extracts.

With this principle in mind, I have prepared concentrated extracts and have preserved these with glycerin for use with the scratch method. In many comparative tests with the two methods the results with all of the extracts thus far examined have been diagnostically the same.

On account of this equality of the two methods as to the results the choice between them will be made with regard to their differences in other respects. Let us examine these differences.

1 Undoubtedly, fewer constitutional effects are produced with the scratch technique than with the intracutaneous injection method.

2 There is usually less likelihood of a nonspecific reaction with the scratch method properly performed than with the intracutaneous method, which is more difficult to carry out properly.

3 The intracutaneous method requires much more paraphernalia and a greater preparation of them than is needed for the simple scratch technique.

4 The proper interpretation of the reactions obtained with the intracutaneous technique calls for decidedly more experience than is usually necessary for the reading of the scratch reactions.

On account of all of these four considerations the scratch method will usually be preferred by physicians who see only few cases of allergic disease and whose patients, for one reason or another, are not able to consult an experienced allergist.\*

<sup>1</sup>Fineman, A. H. J. Immunol. 11: 465, 1926.

\*The allergic specialist is permanently established in medicine. The proper handling of the allergies requires much more than a knowledge of skin testing. But as with other medical specialties it is important that all medical men be able to make a preliminary diagnosis.

On the other hand, the intracutaneous technic has certain important advantages over the scratch method it is cleaner, for the large scale testing by the specialist it is much quicker, it is not necessary to keep the patient's arm in a fixed position, the results can be read in much shorter time (five to ten minutes), there is less scarring of the skin. Furthermore, the intracutaneous method allows testing with different dilutions of the extracts, and there is also the advantage that the same extract can be used for treatment as well as for testing.

Finally, the intracutaneous technic is indispensable for the indirect method of Walzer, which I shall describe presently, and for testing with "autogenous" extracts of dust collected from the patient's living or work room, which are not strong enough for scratch testing.

The most disturbing effect of the nonspecific skin reaction with the intracutaneous technic is, no doubt, seen in the tests for horse serum sensitivity that are made previous to the administration of therapeutic horse serum. If the patient has previously had even a small injection of the serum, as in toxin-antitoxin immunization, he may exhibit an acquired skin sensitivity which does not indicate a constitutional sensitiveness and which should not be taken as a contraindication to the administration of the serum.

In such a situation, the ophthalmic test is a much more reliable one for the existence of the dangerous atopic hypersensitiveness to serum.

#### THE OPHTHALMIC TEST

This test is in some disrepute, possibly on account of the serious damage to the eye which has been reported when the test was applied with tuberculin in tuberculin sensitive persons.

The tuberculin reaction is inflammatory and appears after some hours, it cannot be controlled by any known means. The atopic ophthalmic reaction, on the contrary, is limited to congestion and edema, it appears within a few minutes, and it can usually be immediately controlled with the local application of epinephrin.

In testing the eye for horse serum sensitivity one drop of a 1-10 dilution of horse serum (Park uses the undiluted serum) may be dropped in the lower sac and if there is no congestion or itching after ten minutes the presence of atopic sensitivity to the serum can be excluded and the serum can be administered.

The tests that have been described are all tests of reaginic sensitivity, that is, the positive reactions usually depend on the presence in the blood of the subject of the peculiar human antibodies known as atopic reagents. This circumstance gave the foundation upon which the indirect method of skin testing, which I am about to describe, was developed by Matthew Walzer.

#### THE INDIRECT TEST

In some circumstances it is impossible or inadvisable to test the subject directly. In such case the indirect method of Walzer can be used with great advantage. Walzer makes use of the Prausnitz Kuestner technic as follows:

One twentieth of a cubic centimeter of the patient's sterile serum is injected intracutaneously in 36 sites in the upper arms of a substitute, usually a relative, who should not be atopic. After three to seven days these sensitized sites are tested by the intracutaneous injection of the various extracts and at the same time control injections of the same extracts are made in unsensitized sites. If the reaction in the sensitized site is in any degree greater than that in the unsensitized skin this result is taken as an indication of a corresponding sensitivity in the patient, it shows that the patient's blood contains reagins specific for the material used in the test.

This test is plainly superior to any mode of direct test excepting the clinical test, which is carried out by exposing the patient to natural contact with the material as by inhalation or ingestion. Actually the indirect test is the ultimate criterion of the specificity of a cutaneous or ophthalmic reaction.

The control is practically perfect and even slight differences in the reaction in the sensitized and unsensitized skin are diagnostically significant. Walzer states, for example, that if the test in the unsensitized skin produces no wheal or erythema whereas the sensitized site shows erythema without wheal formation this result can be accepted as a specific reaction.

#### INDICATIONS FOR THE USE OF INDIRECT TESTING

Some of the indications given by Walzer are

1 Abnormal skin conditions of the patient such as, chronic atopic eczema, ichthyotic skins, urticaria, dermatographism and the presence of contagious skin infection (impetigo)

2 Constant severe asthma necessitating the continual use of epinephrin. If the patient is under the influence of epinephrin, a cutaneous reactivity may be suppressed by the drug.

3 Extreme sensitivity where constitutional reactions might result from the direct tests

4 The presence of other severe illness

5 Antipathy to direct test on the part of the patient or his relatives

6 Inability of the patient to come to the physician's office

7 The desire to test the genuineness (specificity) of an unusual number of positive reactions elicited by direct testing

The indirect test has been suggested also by J. Alexander Clarke as a test of reaginic horse serum sensitivity previous to the administration of therapeutic horse serum.

The allergic diseases that are due to a reaginic sensitivity are all subject to a Mendelian hereditary influence, which determines the percentage incidence of this kind of allergy in the offspring, the age of onset of the symptoms, and the clinical form of the allergy.

#### CONTACT DERMATITIS

There is another form of allergic disease which is not subject to a special hereditary influence and in which no antibodies have yet been demonstrated. This form has been called "dermatitis venenata" but is now known by the better name "contact dermatitis."

The list of the known excitants of "contact dermatitis" is large and it is still growing. Beside the well known causes of "dermatitis venenata," poison ivy, poison oak, poison sumac, primrose and others, there are certain dyes used for coloring furs such as uisol, the metals arsenic, nickel, lead and mercury and a host of miscellaneous materials such as printer's ink, iodoform adhesive plaster, formalin, furniture polish, and others. There are also many reported instances of dermatitis due to local contact with various cereals, vegetables and pollens.

A recent study of ragweed pollen dermatitis has shown that the excitant of the condition is not the exciting cause of hay fever in the pollen but the oil of the pollen. This result suggests that contact dermatitis caused by cereals and vegetables may be due to the oils in these materials. We are continuing the investigation of this question and are in need of clinical material of this kind. We should, therefore, be most thankful if any of you who see dermatitis that seems to be caused by direct contact with some vegetable or cereal would test and possibly treat such cases with the oil of the respective material which I should be glad to send you upon request.

The test with the excitants of contact dermatitis is not made by injection or with the scratch technique. Cooke has found that the intracutaneous injection of poison ivy extract fails to produce either a wheal or the typical lesion of ivy sensitiveness.

The cutaneous test for contact dermatitis is made by applying the suspected material to the uninjured surface of the skin and leaving it there for one to five days (J. Jadassohn).

The circumstances under which a person may have natural contact with the excitant of contact dermatitis differ greatly with the different substances, it is very rarely if ever that this lesion follows the ingestion of the excitant, although such excitants frequently occur in food materials (cereals and vegetables), contact with particles of the excitant which are carried by the wind often takes place, as in pollen dermatitis and no doubt also in ivy sensitivity, perhaps the most frequent manner of contact is that had by handling or accidentally touching the material in which is the exciting substance.

It is evident from the foregoing that many a sensitive person may not be known as such because the natural contact which he has with the particular material is not sufficient to induce a reaction, in such cases the existence of the sensitivity must be determined by testing.

The incidence of the sensitivity of contact dermatitis as determined by deliberate testing differs with the different materials. According to Spain, about 60 per cent of persons over three years of age in New York City exhibit sensitivity to poison ivy by test, and H. W. Struss was able to sensitize over 70 per cent of newborn by application of an ivy extract to the skin. Brown, Milford and Coca report that 15 per cent of all persons are sensitive to the oil of ragweed pollen. Bloch states that 100 per cent of human beings can be sensitized with an extract of primrose.

These high percentages compared with that of the inherited group, about 7 according to Spain and Cooke indicate that contact dermatitis is not subject to the hereditary influence which controls the hypersensitiveness of the asthma,

hay fever, eczema group, and this conclusion is confirmed by the personal observation that the incidence of contact dermatitis is the same among asthmatics or hay fever subjects as it is among persons free from these conditions

In the light of the foregoing discussion of the classification of allergic diseases, it is instructive to consider the differences in the etiology, diagnosis, and treatment of the two forms of allergic disease of the skin, the hereditary eczema and the nonhereditary contact dermatitis

For convenience, I have arranged these differences in tabular form in Table I

TABLE I

ATOPIC ECZEMA	CONTACT DERMATITIS
HEREDITARY	NONHEREDITARY
Hereditary abnormality of skin	No hereditary abnormality of skin
Frequently mediated with reagents	No reagents
Water soluble antigenic excitant	Nonantigenic excitant often soluble in fat solvents
Acquired by ingestion	Acquired by surface contact
Skin test by injection or scratch	Skin test by surface application

The specific treatment of contact dermatitis is similar in principle to that used in the atopic conditions (asthma and hay fever), that is, the injection of the specific excitant. It has been resorted to chiefly in cases of sensitiveness to the water insoluble excitant of poison ivy and the most satisfactory menstruum has been found to be sterile almond oil. Two or three injections of this preparation seem to be sufficient in most cases to establish the desired tolerance.

Similar results are being obtained in pollen dermatitis with pollen oil dissolved in the same menstruum.

In conclusion, it may be well to recapitulate the chief points of this paper as follows: leaving out of consideration serum disease, which occurs under artificial conditions, there are two distinct categories of allergic disease, namely the atopic diseases of asthma, hay fever, eczema and others and, contact dermatitis. These two categories differ in their etiology, in their mechanism, and in the methods employed in the identification of their respective excitants. Therefore, they require the use of aqueous extracts with the intracutaneous or

## A-V DISSOCIATION\*

ERNEST BLOOMFIELD ZEISLER, M D , CHICAGO, ILL

THE interpretation of curves showing A-V dissociation presents many difficulties which have not been thoroughly evaluated in the past. Furthermore several closely related questions are involved, such as reciprocal beating of the heart, the supernormal phase, prolongation of the P-R conduction interval, and variation of the P-P intervals in A-V block. The present report is made in an endeavor to clarify the analysis of such curves. In the following presentation I have attempted to treat the subject of A-V dissociation in logical order, the facts presented being derived from the study of original curves which were correlated with an analysis of previous reports.

A-V dissociation denotes the condition in which the auricles and the ventricles beat independently of each other, that is, the auricles and the ventricles are controlled by different pacemakers. If no ventricular beat is a response to the pacemaker controlling the auricles, and vice versa, then there is complete dissociation, if some but not all of the ventricular beats are in response to the same pacemaker controlling the auricles, or vice versa, then there is incomplete dissociation or dissociation with interference.

The best known and most common example of complete A-V dissociation is in complete A-V block without any other disturbance, the auricles are controlled by the S-A node, the ventricles by a pacemaker in the A-V node or in the crux commune, and none of the impulses pass from the auricles to the ventricles or vice versa. A-V dissociation and complete A-V block are, however, by no means synonymous, although complete A-V block is always accompanied by complete A-V dissociation, the converse is by no means true. It is necessary to distinguish also between primary or essential A-V block and secondary or adventitious A-V block. By primary A-V block is meant A-V block due to prolongation of the refractory period of the A-V node or crux commune, such as in the blocking of a normal auricular impulse in normal sinus rhythm. By adventitious A-V block is meant A-V block due not to prolongation of the refractory period but to the fact that the impulse reaches the A-V node or crux commune during its normal cyclic refractory period, such as in the blocking of a very early auricular extrasystole,<sup>28</sup> and in auricular fibrillation and flutter. Practically all ventricular extrasystoles are blocked from the auricles, that is, such an extrasystole is rarely conducted back to the auricles †. The same is true in most cases of ventricular paroxysmal tachycardia, so that the auricles beat in response to their own pacemaker. Consequently there is momentary A-V dissociation in ventricular extrasystoles and in very early auricular extrasystoles, and complete A-V dissociation

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†When it is conducted backward it is a retrograde ventricular extrasystole.



in ventricular paroxysmal tachycardia. Aside from these obvious instances the study of A-V dissociation may be divided into two parts without and with primary A-V block.

#### A-V DISSOCIATION WITHOUT PRIMARY A-V BLOCK

In this condition the auricles are controlled by the S-A node and the ventricles by the A-V node. If we let  $A$  and  $V$  represent the auricular and ventricular rates respectively,\* then it is obvious that  $A \leq V$  for if  $A$  were greater than  $V$  the auricular impulses would be conducted to the ventricles since there is no primary A-V block.

The principle of the mechanism is best illustrated by the diagram (Fig. 1) of an ideal case. Let  $A$  and  $V$  both be constant  $A = 67$ ,  $V = 75$ , and let  $A_0$  and  $V_0$  be simultaneous. There is a regular sequence  $A_0, A_1, \dots$  of auricular beats

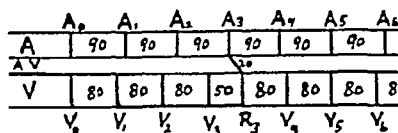


Fig. 1

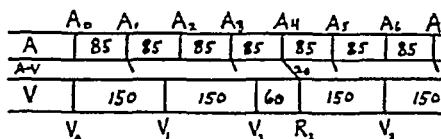


Fig. 2

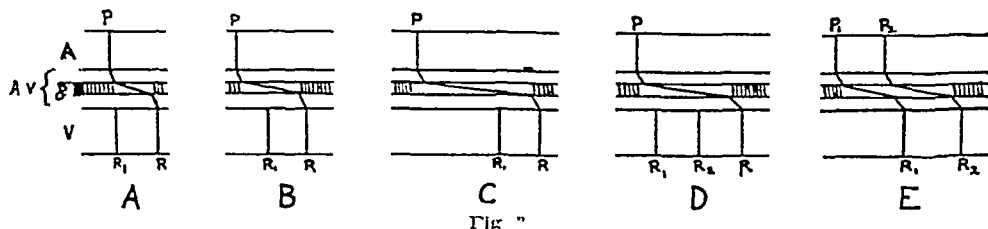


Fig. 3

Fig. 1—Diagram of interference dissociation. Intervals in hundredths of a second.

Fig. 2—Diagram of dissociation with interference in primary 2:1 A-V block.

Fig. 3—Diagram of five types of skip conduction. B is a region of almost complete forward and complete retrograde block. O is just below B.

The impulse  $A_1$  is not conducted because the recovery period  $V_1A_1 = 10$  is too short.<sup>25</sup> Let the minimum recovery period for conduction be 25, then  $A_2$  is not conducted, but  $A_3$  is conducted because its recovery period is  $V_3A_3 = 30$ , which is above the minimum. Let the conduction time of  $A_3$  be 20, there thus results a conducted beat  $R$  with an interval of coupling  $V_3R = 50$ . The passage of the impulse from  $A_3$  through the A-V node annihilates the partially formed nodal impulse, so that  $R_3V_4$  is again 80. The recovery period  $R_3A_4 = 70$ , so that  $A_4$  would be conducted did not  $V_4$  follow  $A_4$  too closely,  $A_5$  and  $V_5$  are again simultaneous, so that if  $A$  and  $V$  remain constant and if there is no change in A-V conductivity this process will continue in cycles, and every sixth ventricular beat will be conducted from the auricles with a fixed coupling interval of 50.

In general, let  $a$  and  $v$  be the AA and VV intervals  $a > v$ , and  $A_0$  and  $V_0$  be simultaneous. Now the recovery period of  $A_k$  is  $V_kA_k = k(a-v)$ , this increases until it is at least as big as the minimum recovery period, since there is no primary block, so that finally there is a conducted beat. Let  $A_m$  be the first conducted

\*These terms  $A$  and  $V$  will be used throughout.

beat, its recovery period is  $m(a-v)$ , let its conduction time be  $c = (n-m)(a-v)$ . Then the interval of coupling is  $i = n(a-v)$ . Thus  $na = nv + i$  and  $ma + c = mv - i$ , so that  $A_n$  and  $V_n$  are simultaneous, consequently every  $n$ th auricular beat is conducted and there are repeated cycles of  $n$  nodal ventricular beats followed by one conducted ventricular beat with fixed coupling. Here there is complete A-V dissociation except for the periodically conducted impulses, because the conducted impulse annihilates the partially formed nodal impulse it interferes with the nodal rhythm and shifts it, for this reason this condition, first described by Mobitz,<sup>15 16 17</sup> was called interference-dissociation. We note that  $i = c - m(a-v)$ , the interval of coupling is at least the sum of the conduction time and the minimum recovery period. Normally the minimum conduction time is 12 to 20, and requires a recovery period of about 30, so that the sum of these two is 42 to 50, as the recovery period decreases the required conduction time increases without much change in their sum,<sup>25</sup> hence the coupling interval  $i$  has a relatively long minimum, say 40 to 50, in fact, the minimum of the coupling interval varies as the absolutely refractory period of the A-V node plus the conduction time from the A-V node to the ventricles.

If we depart from the ideal case we find the principle of the mechanism unaltered. Slight sinus or nodal arrhythmia changes the sequence of events, so that the intervals  $a, v, c, i$  are not constant but vary slightly. Similarly, changes in tonus of the vagi and sympathetics alter  $a, v, c$ , so that again the intervals vary. If we do not assume  $A_0$  and  $V_0$  simultaneous the numerical relations are altered. The result is that there is no exact periodicity of cycles, but there is still dissociation with occasional interference.

The same mechanism may give rise to complete A-V dissociation, for suppose  $A = V$  and  $A_0$  precedes or follows  $V_0$  by a short interval, for example 10, then  $A_m$  precedes or follows  $V_m$  by 10 and is not conducted, so that the dissociation is complete. Thus, of course, occurs very rarely, it is illustrated in Leads I and II of Fig 11 which will be discussed later. As stated before we must have  $A \leq V$ , whereas normally  $A > V$ . We may have  $A \leq V$  because of auricular bradycardia (which may be due to sinus bradycardia or sinoauricular block) or because of nodal tachycardia or because  $A$  and  $V$  are both increased or decreased but in different amounts. We present here two illustrative cases of interference-dissociation, the first is due to sinus bradycardia ( $A = 45, V = 65$ ) and the second to nodal tachycardia ( $A = 96, V = 99$ ).

CASE 1—A curve taken before treatment shows sinus arrhythmia, rate 88, P-R = 0.16 second. Fig 4 was taken after the patient had received 60 minims daily of tincture of digitalis for eight weeks, Fig 5 was taken the same day as Fig 4 and five minutes after subcutaneous injection of one-fiftieth grain of atropin sulphate. Then digitalis was stopped and nine days later an electrocardiogram showed sinus rhythm rate 86, P-R = 0.20 second.

In Figs 4 and 5 are shown the time intervals in hundredths of a second, the oblique lines merely show the P-R and R-P intervals without connoting conduction. This method of analysis has the advantage of showing all the time intervals without committing itself to any particular interpretation.

In Lead I of Fig 4 the P-waves are fairly regular with an average P-P of



decreases, as is to be expected.<sup>28</sup> The auricular impulses are conducted whenever they happen to come long enough after the preceding R so that the A-V node is no longer absolutely refractory when they reach it. The result in this case is 3 2 partial A-V block,\* but this block is purely adventitious as shown by the normal P-R of 16 when the recovery period is long enough. In Lead I the beats R<sub>2</sub>, R<sub>4</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>9</sub>, R<sub>11</sub> are escaped nodal beats. In Fig 5 all the P-waves are conducted to the ventricles, there is not even any adventitious block, but due to the auricular bradycardia every second ventricular beat is an escaped nodal beat.

TABLE I

R-P	60	58	57	57	56	56	30	30	27	26	26	26	24	<18
P-R	16	15	16	16	16	16	22	26	26	27	28	28	28	-

The following explanation of this case is offered. In Fig 4 digitalis has produced an auricular bradycardia, without any effect on the A-V conduction time. Atropin had very little effect on the auricular rate, merely increasing it from 44 to 48, it also had very little effect on the A-V conduction time (which was already normal), the change from Fig 4 to Fig 5 is due entirely to the increased rate of the nodal rhythm. After nine days without digitalis the mechanism returned to normal, so that the arrhythmia was surely due to digitalis, the main action of which was to produce auricular bradycardia.

CASE 2—The curve of Fig 6 was taken after the patient had received digitalis for some time. There is a slight sinus arrhythmia, A = 96, except for four R-R intervals the ventricular rate is nearly constant, V = 99. The short R-R intervals are due to conducted beats as indicated. All the other P waves are blocked because they happen to come either too early or too late. In Lead I, P<sub>6</sub> to P<sub>10</sub> come too early, R-P ≤ 7, and they are adventitiously blocked, P<sub>1</sub> to P<sub>5</sub> come too late, so that the next R come before they have time to be conducted. For similar reasons there is no retrograde conduction. There is no primary block in this case. After three days without digitalis there was sinus rhythm with normal P-R intervals, so that the arrhythmia was certainly due to digitalis, which acted by producing nodal tachycardia.

#### CLINICAL DISCUSSION

This type of interference dissociation is certainly uncommon, as shown by its incidence only twice in several thousand electrocardiograms observed. It is especially likely to occur in digitalized hearts. Without the electrocardiogram the diagnosis is practically not possible, it is, however, sometimes important. For example, in Case 1 the pulse or the apex beat at the time Fig 5 was taken would very likely give the impression of pulsus bigeminus with auricular bradycardia and ventricular extrasystoles due to digitalis, and interpreted as a danger signal of severe digitalis intoxication. With the electrocardiogram the diagnosis is not difficult though it is usually missed. Fig 6 was published as a case of complete A-V block, and the short couplings were left unexplained.<sup>4</sup> Fig 5 is the type of

\* i. e. every third auricular impulse fails to be conducted to the ventricles

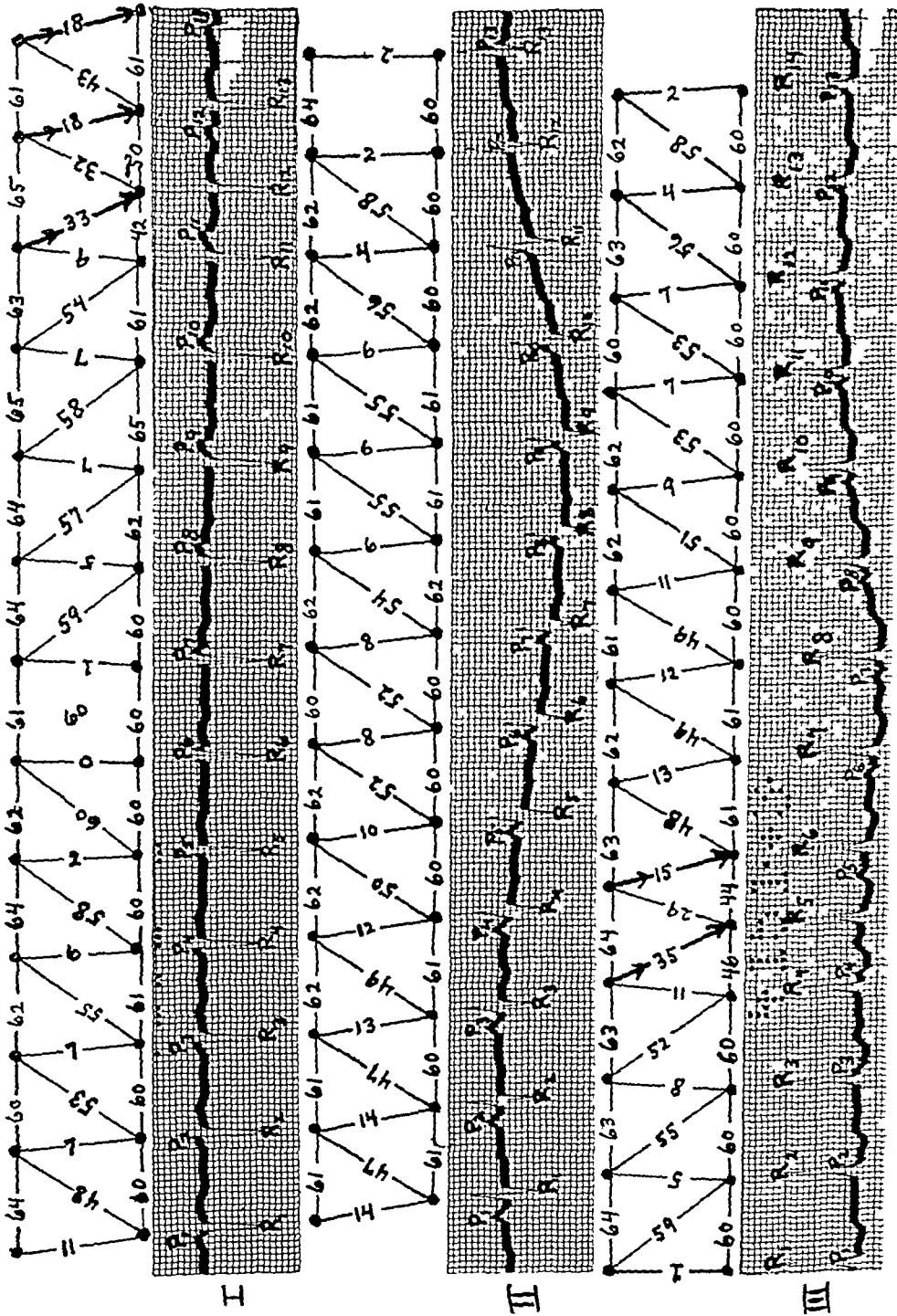


Fig 6—Case 2 Sinus rhythm, auricular tachycardia of 99, nodal tachycardia of 99 No primary A-V block Interference-dissociation, almost complete

curve usually misinterpreted as reciprocal beating of the heart. In general, it may be said that in a regular series of supraventricular complexes interrupted by shorter R-R intervals dissociation with interference should be considered, and is especially likely to occur during digitalis administration.

*Reciprocal Beating of the Heart*—Reciprocal beating of the heart is the condition in which an A-V nodal impulse is conducted back to the auricles, causing their contraction, and then this auricular impulse is conducted forward again to the ventricles and causes them to contract. A priori such an occurrence seems very unlikely indeed, and only a clear-cut example should be accepted as evidence for its occurrence. I have studied a number of cases reported in the literature as reciprocal beating,<sup>3, 5, 6, 7, 8, 24, 25, 27</sup> the reproductions of many of these curves are too indistinct for careful analysis. Before the mechanism can be called reciprocal beating it must be demonstrated that the P-wave between the coupled R-waves is actually retrograde, for which it is by no means sufficient that P be negative or deformed (see discussion of Fig. 11). Many of the reported curves are readily explained as, and probably are, dissociation with interference.\*

In true reciprocal beating the impulse back from A to V probably does not retrace the path by which it has just come from V to A but goes back over fibers which were quiescent during the retrograde conduction.<sup>14</sup>

#### A-V DISSOCIATION WITH PRIMARY A-V BLOCK

Primary A-V block may properly be considered as of five degrees which may be tabulated according to the number of auricular impulses which are completely blocked and those conducted.

TABLE II

DEGREE	AURICULAR IMPULSES COMPLETELY BLOCKED	AURICULAR IMPULSES CONDUCTED
first	none	all
second	occasional	most
third	many	many
fourth	most	occasional
fifth	all	none

It is seen that the first and fifth degrees and the second and fourth degrees are counterparts.

*First Degree A-V Block*—When all the auricular impulses are conducted to the ventricles, even though P-R is prolonged, there is no dissociation except if the nodal rhythm is more rapid than the auricular, that is  $V > A$ , in this case the result is the same as interference dissociation, with perhaps longer P-R for the conducted beats, it will thus appear like Fig. 5.

*Second Degree A-V Block*—In second degree block there may or may not be

\*Of fourteen curves ten are almost certainly interference dissociation, mostly with auricular  $A < V$ . White<sup>24</sup> gives Fig. 14 with  $A = 70$ ,  $V = 75$ , and Fig. 15 with  $A = 40$ , and Fig. 2 with  $A = 50$ ,  $V = 55$ . Blumgart and Gargill give Fig. 1 with  $A = 48$ ,  $V = 55$ , which is actually reciprocal beating. Of the remaining four curves one appears doubtful but is probably actually reciprocal beating. The remaining three curves are without doubt reciprocal beating. There is one additional case of reciprocal beating which was missed by the author who published the curve.

an occasional escaped beat after an interval  $p$  depending on three things (1) the auricular rate  $A$ , (2) the conduction time  $P-R$ , (3) the rate  $V$  of the center just below the point of block. It should be noted that  $A$  and  $V$  depend on the two pace makers concerned, and also on the tonus of the cardiac nerves at that time. If  $pV = 60$  then the lower center escapes, if  $pV < 60$  there is no escape. Such an escaped beat is usually not conducted back to the auricles so that there is a momentary dissociation.

*Fifth Degree A-V Block*—In complete A-V block the auricles respond to the S-A node and the ventricles to a pacemaker just below the point of block in the A-V node or the crux commune with so-called idioventricular rhythm. The result is complete dissociation. Ordinarily  $A > V$  but it may occur with  $A \leq V$ .

CASE 3—In Fig. 7 is shown Lead II of a case of complete A-V block.  $A = 78$ ,  $V = 47$ . In addition to complete dissociation this curve shows a phenomenon of fairly common occurrence but frequently overlooked. The P-P intervals are of two kinds: those like  $P_1P_2$  and  $P_2P_3$  which contain an R, and those like  $P_3P_4$  and  $P_5P_6$  which contain no R. The nine intervals of the first kind measure 67 to 76 and average  $72\frac{1}{2}$ , the five intervals of the second kind measure 79 to 88 and average 84. Hence the maximum, the minimum, and the average interval of the second kind are 12 each longer than the maximum, the minimum, and the average interval respectively of the first kind, and the minimum 79 of the second kind is longer than the maximum 76 of the first kind. This cannot be accidental, the same phenomenon is seen in many cases of complete and also of partial A-V block,<sup>9, 10, 19, 22, 26</sup> although it is often absent.

That the shortening of the P-P intervals which contain an R is not entirely dependent on vagus influence is demonstrated by its persistence, though to a lesser degree, after injection of atropin.<sup>9</sup> The most reasonable explanation is as follows: in ventricular bradycardia the coronary blood flow is improved by ventricular systole, the circulation of the S-A node is improved, so that the S-A node builds up and discharges its next impulse sooner.<sup>10, 22</sup> Some authors place the improvement in S-A conduction rather than in the S-A node.<sup>11, 19</sup>

*Third Degree A-V Block*—In the absence of primary A-V block there can be no permanent A-V dissociation if  $A > V$ , but it can occur with primary A-V block. For example, let there be primary 2:1 block,  $A = 71$ ,  $V = 40$ . The diagram of Fig. 2 shows the mechanism. Alternate impulses  $A_1, A_3, A_5$ , etc., are blocked in the A-V node because of the primary 2:1 block, otherwise the mechanism is the same as in Fig. 1, including the periodicity and the fixed, relatively long coupling.

CASE 4—Let us first examine Leads I and II of Fig. 11. There are three possible explanations: (a) nodal rhythm, rate 91, with retrograde conduction, (b) sinus rhythm, rate 91,  $P-R = 54$ , (c) complete A-V dissociation,  $A = V = 91$  with primary complete A-V block, or without any primary A-V block.

From Leads I and II of Fig. 11 we cannot determine which of the three interpretations is correct, though the deformed P of Lead I and the inverted P of Lead II favor (a). But in Lead III we see the same regular auricular rhythm with a quite irregular ventricular rhythm, this makes it quite improbable that the auricular rhythm is dependent on the nodal, and is strongly against (a) though it does not disprove it, for there might be forward block from the node but no retrograde block.

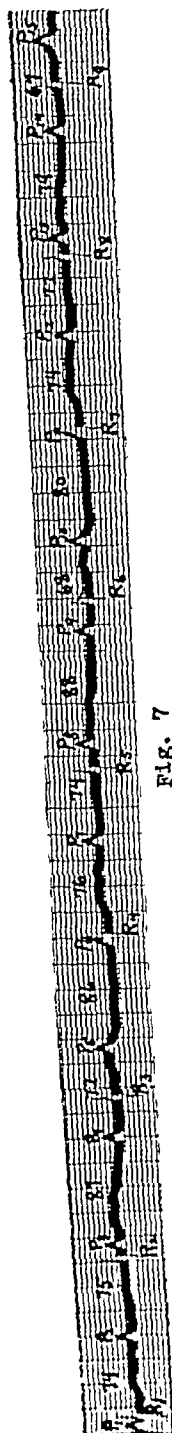


Fig. 7

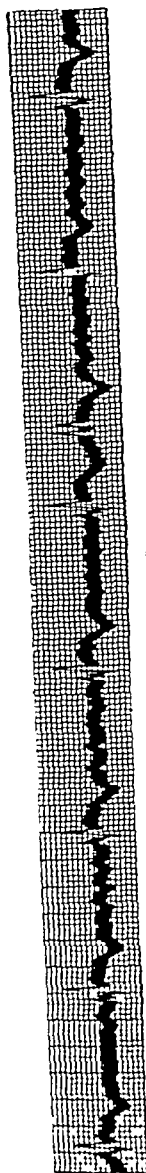


Fig. 8

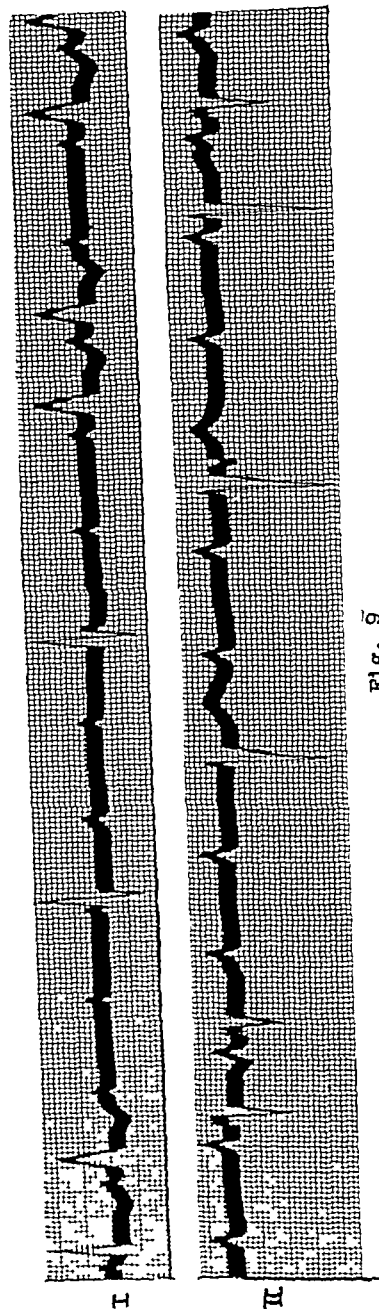


Fig. 9

Complete A-V dissociation in complete A-V block Variation of P-P intervals due to ventricular systole  
 Auricular fibrillation fourth degree A-V block  
 Fourth degree A-V block, Leads I, II Aberrant conduction Retrograde conduction

I 7 — C<sub>100</sub> 1  
 II 8 — C<sub>100</sub> 2  
 III 9 — C<sub>100</sub> 3



In Fig 10 are shown three previous curves from the same patient taken at intervals, the top curve first. These curves clearly show sinus rhythm. There is a gradual change in the P-waves, P becoming diphasic in Lead I and negative in Leads II and III, so that the P-waves of Fig 11 are not much different from those in the last curve of Fig 10. This indicates more or less conclusively that the P-waves of Fig 11 are not retrograde, so that explanation (a) is invalid. The marked irregularity of the ventricular beats in Lead III of Fig 11 and in Fig 12 eliminates (c), so that we are left with (b).

To explain Fig 11\* and Fig 12 it is unnecessary to assume the presence of any escaped beats, and the marked variation of the longer R-R intervals from 112 to 125 speaks strongly against escape. The regularity of Table III also is in favor of conduction.

TABLE III

P P	73	70	R P	39	39	39	38	38	38	38	37	19	18	17	17	16	16	16	<14 =
P R	32	35	P R	52	53	53	54	54	54	55	57	-	-	65	-	62	-	-	-

In Fig 10 the P-R of the three curves are 23, 22, and 23, and in Fig 12, Lead I the P-R is 32 after a full rest of P-P = 70, this is evidence of some degree of primary A-V block. Fig 12 represents sinus rhythm and third degree A-V block with no escaped beats, the block is mostly 3:2 but is occasionally 4:3 and 2:1. In Fig 12 there is a conduction interval of 65, namely  $P_6R_7 = 65$ , with  $R_6P_9 = 17$ . The absence of dropped beats in Leads I and II of Fig 11 is due to a lesser degree of block at that time, as shown by the shorter P-R in Fig 11, Lead I in spite of shorter R-P.

In passing I merely call attention to an instance of sinoauricular block in Lead III of Fig 11, in which  $P_6$  is dropped.

*Fourth Degree A-V Block*—The degree of A-V block may be great enough so that for long intervals no beats are conducted, and only occasionally is there conduction.

**CASE 5**—In Fig 8 is shown a case of fourth degree block. The auricles are fibrillating, at a rate of about 460, almost all the auricular impulses are blocked, and the ventricles respond to a pacemaker in the crux commune with a rate of 50. Only one auricular impulse is conducted.

**CASE 6**—(A male of seventy-four years.) For forty-four years this man had had a pulse rate of about 30, and for the past three years he had Stokes-Adams attacks. In Fig 9 it is seen that only five P-waves are conducted to the ventricles in Lead I, and only three in Lead II, there is, therefore, a high degree of primary A-V block. The abnormality of the conducted ventricular complexes is doubtless due to aberrant conduction in the bundle. A-V conduction, when it occurs, is due to a temporary improvement (circulatory or otherwise) in the diseased portion of the bundle, and the conduction of a second beat is probably partly due to the local circulatory improvement caused by the ventricular systole preceding. This view is favored by the history of Stokes-Adams attacks, which are due to temporary relapses in the diseased portion of the bundle.

\*To conserve space only part of strips I and II (for which Table III is made) are reproduced

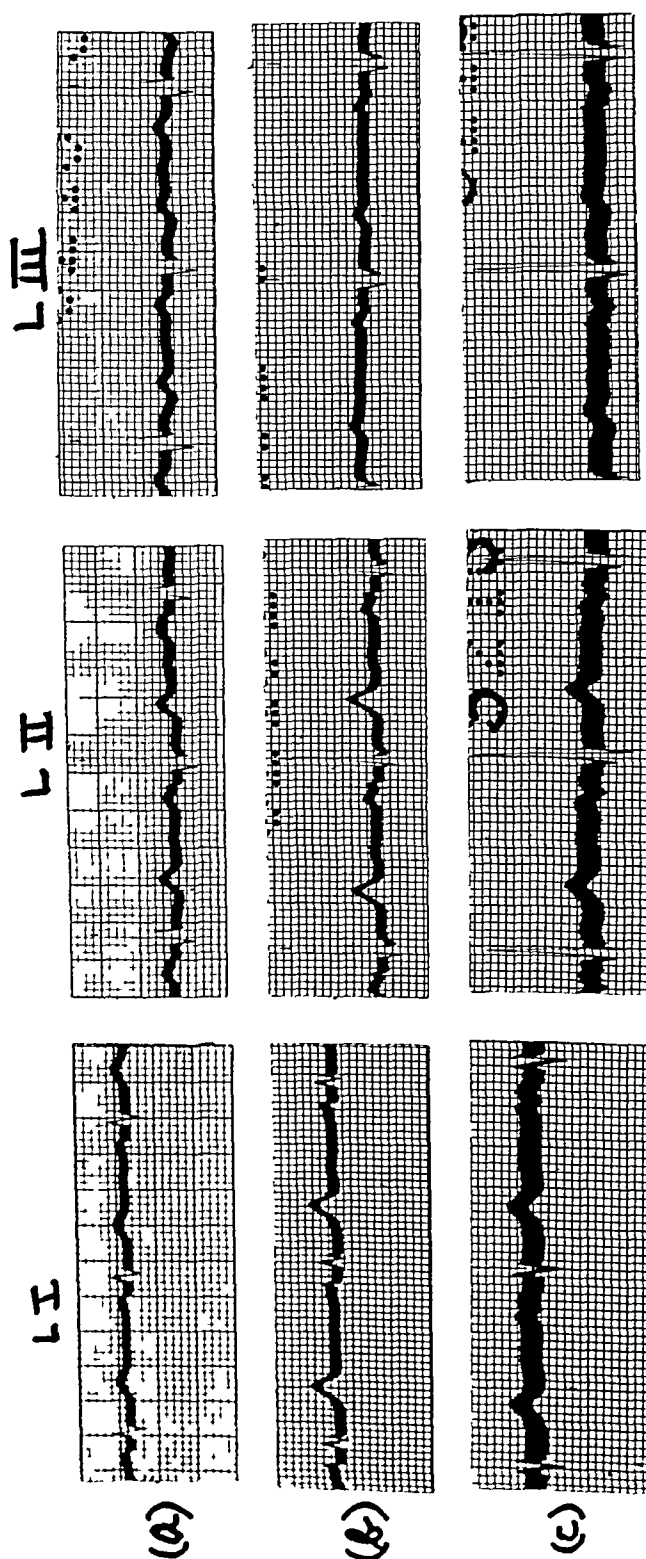


Fig. 10

Fig. 10 — Case 1 Three curves at intervals of several months, the top curve first Leads I II III from left to right First degree A-V block Note gradual change in P-waves

An interesting phenomenon is the retrograde conduction seen in Lead II of Fig 9 This was frequently repeated in unpublished parts of the curve It seems that retrograde conduction is not at all uncommon in high degrees of A-V block 2"

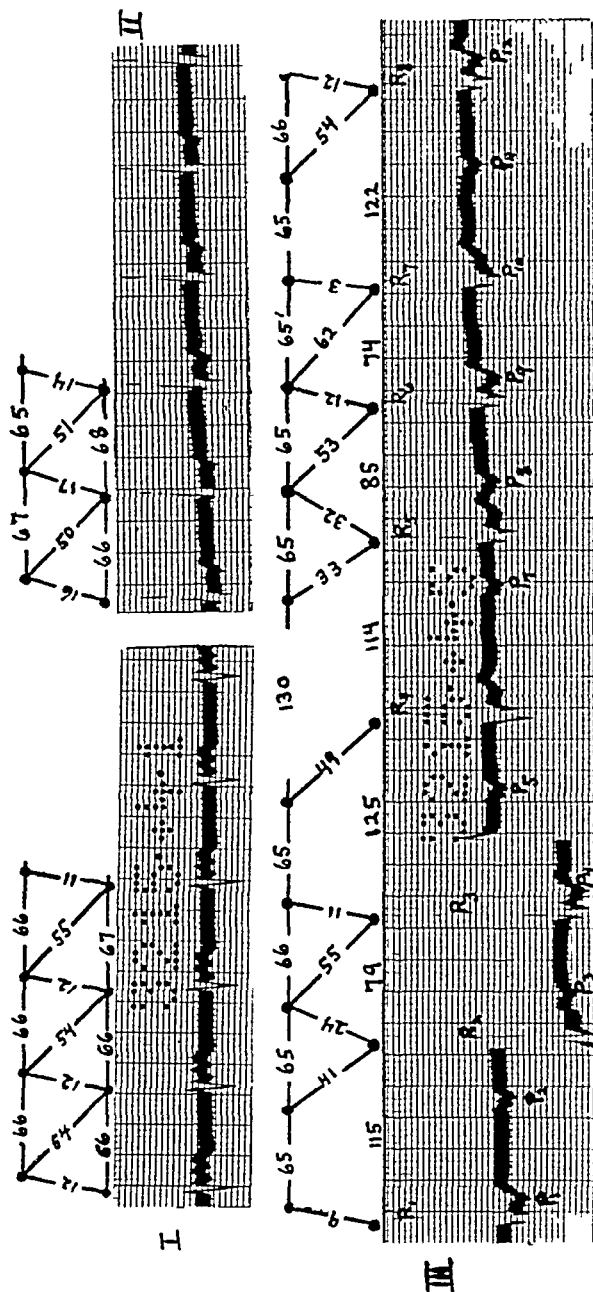


Fig. 11

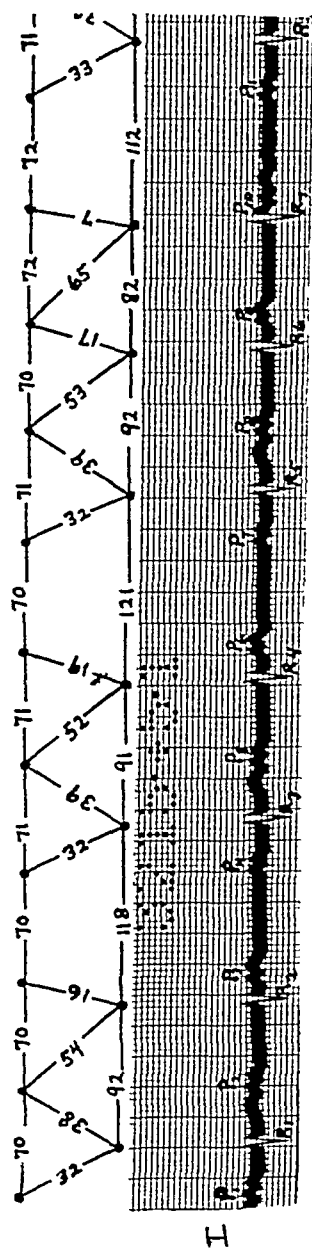


Fig. 12

Fig 11—Case 1 Sinus rhythm First and second degree A-V block No escaped beats Sinus rhythm block at P<sub>4</sub> in Lead III  
Fig 12—Case 4 Sinus rhythm Third degree A-V block (1 3 and 3 2), Lead I Conduction interval P<sub>2</sub>R<sub>2</sub> of 0.65 second

#### TOPICS RELATED TO DISCUSSION OF A-V DISSOCIATION

*The Supernormal Phase*—There are a number of recorded cases in the literature of third or fourth degree A-V block in which only auricular impulses with short recovery periods are conducted, while those with longer recovery periods

are blocked. This apparently paradoxical occurrence is explained by Lewis and Master<sup>13</sup> by assuming a supernormal phase, according to which the passage of an impulse through the A-V node is followed by a short period of supernormal conductivity, so that an auricular impulse very early in diastole is conducted, whereas one falling later in diastole falls outside the supernormal phase and so is not conducted. Ashman and Herrmann<sup>1</sup> also give curves showing this phenomenon.

It is necessary first of all to state precisely what is meant by a supernormal phase. In the discussion of complete A-V block it was mentioned that in ventricular bradycardia the blood circulation of the S-A node is improved by ventricular systole, the same is probably true of the A-V node and bundle, this might very well increase A-V conductivity. Changes in tonus of the cardiac nerves may also increase A-V conductivity. Hence an apparent supernormal phase may be due to improved coronary blood-flow or to changes in tonus of the cardiac nerves such as perhaps occurs in Figs 8 and 9, but this cannot properly be called a supernormal phase, a term which should be applied only to a phase of increased conductivity due solely to the passage of a previous impulse over the tissue in question, as in the case of an isolated nerve-fiber. In neither of these cases have Lewis and Master excluded changes in tonus of the cardiac nerves, and in their second case they may be dealing with ventricular extrasystoles. The occurrence of a true supernormal phase of A-V conductivity has not been demonstrated.

*Very Long P-R Intervals*—The question has been frequently raised as to how long the P-R interval may be. Barker and Bridgman<sup>2</sup> state that there is no upper limit to the possible length of P-R, this statement is unquestionably wrong, for there can be no doubt that if an auricular impulse is not conducted within say an hour it will not be conducted at all, so that P-R can never be greater than one hour. The important question is, however, not how long we think P-R may be but how long it actually is, in other words, what is the longest P-R ever recorded? Before we can speak of a P-R interval we must be sure that the R is actually due to conduction of the impulse from P, and this is frequently not easy to decide.

There are many recorded P-R intervals of 40 to 50 (in hundredths of a second) though they are uncommon. In Case 4, Fig 12, we have a P-R interval of 65. The only longer unquestionable P-R conduction intervals I have seen are in cases of Thayer<sup>20</sup> and Herrmann and Ashman,<sup>12</sup> in which there are P-R intervals as long as 73. But several authors have reported longer P-R intervals, ranging from 80 to 103\*.

\*I should like to discuss some of these cases in a little detail. Barker and Bridgman<sup>2</sup> believe they have P-R intervals of 90 to 103. Careful analysis of their curve demonstrates that the longest actual P-R interval is 43, the R which they say are conducted after the intervals 90 and 103 are clearly escaped beats following block of the preceding auricular impulses. Thayer<sup>20</sup> reports a case with P-R interval of 102. In his electrocardiograms the P-R intervals vary from 64 to 73, on the same day on a polygram is marked an a-c interval of 102 followed two beats later by an a-c of 60, the long a-c interval is quite unconvincing so that the longest conduction time is 73. Wenckebach and Winterberg<sup>2</sup> show a curve in which they believe there is a P-R interval of 96, all alike. There is obviously more than moderate A-V block, present, inasmuch as there are twenty-two P waves and only twelve R-waves. With such a degree of A-V block it is unlikely recovery periods of from 45 to 54, whereas in normal sinus rhythm in the normal heart the recovery period<sup>21</sup> is 65 and only uncommonly shows a conduction time of as short as 11. A priori it seems unlikely also that a P wave should be conducted to an R with an intervening R, so-called skip conduction, but this objection alone is not valid as we shall see. There are two other possible explanations of their curve both I think more likely than theirs. First, we may be dealing with tachycardia of 94. Secondly, there may be 2 1 A-V block (except at one place) with a maximum cluster reason for assuming skip conduction in their curve so that we cannot accept their long P-R. It would be equally justifiable to assume skip conduction in our Case 6 which would yield us a P-R of 88, namely I R<sub>1</sub> in Lead I of Fig 6.

*Skip-Conduction*—By skip conduction I mean conduction from an auricular impulse P to a ventricular impulse R with an intervening ventricular complex  $R_1$ , as shown in Fig 3. Can this occur, and if so, how? Clearly  $R_1$  cannot originate in the A-V node, with a normal conduction time, for otherwise P would be blocked and could never reach the ventricles to cause R, hence  $R_1$  must originate below a region of block, viz, at a point O in the crux commune (Fig 3), this presupposes a region of block B just above O. It is also clear that  $R_1$  must be discharged from O before P reaches O, and furthermore, that  $R_1$  is not conducted upwards, for otherwise it would meet and block the downcoming P. Since P is to be conducted through O it must reach O a considerable time after O discharges  $R_1$ , which can occur only because P is delayed long enough in B. If then we assume such a region of block B with partial forward and complete retrograde block it is theoretically possible to have skip-conduction as shown in the various segments of Fig 12. That this actually occurs is demonstrated conclusively by Scherf's<sup>18</sup> case in which there is skip-conduction of type (a).

*Crux Commune Block*—With a region of block in the crux commune as discussed above it is clear that the minimum coupling interval  $R_1R$  (Fig 3) varies as the absolutely refractory period of the center O plus the conduction time from O to the ventricles, which is shorter than the conduction time from the A-V node to the ventricles. In this way can be explained cases of A-V dissociation with interference in which the interval of coupling is relatively short, and shorter than that in interference-dissociation. The mechanism is similar to that of interference-dissociation, in analogy to Fig 1 and Fig 2.

*Organic Block*—Before we can conclude that A-V block is organic we must first show that it is primary, then we must be sure that it is not chemical (e g digitalis), and finally we must exclude vagus influence which is best done by injection of atropin.

#### SUMMARY

1 A-V dissociation is the condition in which the auricles and the ventricles are controlled by different pacemakers, if occasionally they respond to the same pacemaker the condition is incomplete dissociation or dissociation with interference, otherwise it is complete dissociation. Complete A-V dissociation and complete A-V block are by no means the same thing.

2 Primary or essential A-V block is block due to prolongation of the refractory period of the A-V node or the bundle, adventitious A-V block is block due not to prolongation of the refractory period but to the fact that the impulse reaches the A-V node or the bundle during its normal cyclic refractory period.

3 A-V dissociation of any degree, even complete, may be present without any primary A-V block, this is interference dissociation, and occurs only if  $\Delta \leq V$ .

4 In a regular series of supraventricular complexes interrupted by shorter R-R intervals dissociation with interference must be considered, especially during the administration of digitalis.

5 Reciprocal beating of the heart is the condition in which an A-V nodal impulse is conducted back to the auricles, and then this auricular impulse is conducted forward again to the ventricles. Most of the curves reported as reciprocal beating are really interference-dissociation. There are actually four or five reported cases of true reciprocal beating.

6 Primary A-V block is divided into five degrees instead of the four degrees heretofore considered

7 In many cases of complete and of partial A-V block, the P-P intervals which contain an R are shorter than those which do not. This may be due to improvement of the circulation to the S-A node by ventricular systole

8 A true supernormal phase of A-V conduction denotes a phase of increased conductivity due solely to the passage of a previous impulse through the A-V node, this has never been conclusively demonstrated to occur

9 Those P-R intervals recorded of from 0.80 second to 1.03 seconds probably do not represent conduction but escaped beats. The longest recorded P-R conduction interval which can be accepted without question is 0.73 second. A case is shown with a P-R conduction interval of 0.65 second

10 Skip conduction is conduction from an auricular impulse to a ventricular impulse with an intervening ventricular complex. Theoretically this is possible with a region of partial forward and complete retrograde block in the crux commune. A case is referred to in which skip-conduction actually occurs

11 Before concluding that A-V block is organic it must be shown that it is primary and not adventitious, that it is not due to digitalis or anoxemia, and finally that it is not due to vagus influence which is best tested by atropin

12 The foregoing discussion permits a classification of A-V dissociation into the following categories (aside from the obvious instances in early auricular extrasystole, ventricular extrasystole, and paroxysmal ventricular tachycardia)

Without primary A-V block

$A < V$  (Auricular bradycardia, nodal tachycardia)—interference-dissociation

rarely  $A = V$ —complete dissociation

With primary A-V block

2nd degree A-V block—escaped beats

3rd and 4th degree A-V block  $\begin{cases} A \neq V & \text{—dissociation with interference} \\ A = V & \text{—complete dissociation} \end{cases}$

5th degree A-V block—complete dissociation

I wish to express my indebtedness to Dr. Louis N. Katz, at whose instigation this research was undertaken, for his stimulating discussion and criticism

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## THE CONTROL OF POLLEN ALLERGY\*

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WHILE sensitization to pollens may be responsible for attacks of pollen dermatitis or eczema or even gastrointestinal manifestations by far the commonest symptoms of pollen allergy are hay fever and asthma. The doctor in the past has given little attention to the treatment of hay fever and asthma due to pollens, because he has found that not much can be done for these sufferers. Even the treatment with pollen extracts, first hailed as a great panacea, was found to fail so often that in many sections it became quite discredited. Indeed, even today it is true that pollen therapy as administered by the majority of physicians is more often disappointing than otherwise.

*Incidence*—When properly applied, pollen therapy will give practically complete relief to two-thirds of seasonal asthmatic and hay fever cases. When properly applied fully 90 per cent will obtain adequate relief, sufficient for them to be entirely satisfied. If these two statements can be substantiated, and I shall have no difficulty in doing so, we are justified in giving some thought to the problem of hay fever and asthma due to pollens. There being between 3 and 4 million such cases in this country, the problem becomes one that touches every man interested in the general practice of medicine. Balyeat gives a rough estimate of 2 per cent of the population suffering from hay fever or asthma. Piness and Miller found, in a careful survey of two populations totaling 4 000 people in the West, that 4.4 per cent of one and 3 per cent of the other were hay fever victims. Cooke and Vander Veer in a similar survey in New York found 3.5 per cent of the population suffering from asthma or hay fever. If one out of thirty in the population at large suffers from hay fever or asthma from one cause or another, allergic therapy must necessarily be of interest to the average physician. Indeed, it has been estimated that one of every fourteen suffers from some form of allergy, and this estimate is decidedly conservative.

The question before us is, what is adequate pollen treatment and how should it be given, by what measures may we obtain the excellent therapeutic results just mentioned?

Inhalant allergy, asthma, hay fever, and vasomotor rhinitis are due to a variety of causes, pollens, house dust, feathers, animal epithelia, oris root, occupational dusts, pyrethrum and other less frequent causes. The appropriate

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treatment necessarily varies depending upon the cause. The chief among these however is the pollens.

The first thing to remember is that pollen allergy is seasonal. It does not persist throughout the year. As usual there are exceptions, such as workers in greenhouses and those who live where pollen is in the air throughout the year, as in Texas and Southern California. But the general rule holds. Always suspect pollens when the symptoms are worse during the warm months of the year.

#### DIAGNOSTIC REQUISITES

What are the requirements for adequate diagnosis of the cause of pollen allergy?

*A Botanical Survey*—First one must know what hay fever plants are indigenous to the territory in which the patient lives and the period of their bloom. This was one of the major problems confronting allergists early in their work. Excellent botanical treatises were available, detailing the distribution of the flora of the country, but they were too general for reliable use in this work. The early tables of distribution, prepared by the manufacturers of pollen extracts were based chiefly upon these general surveys and were in no sense accurate for specific localities. It became necessary to make local botanic surveys throughout the country, paying especial attention to those trees, grasses, and weeds which have in the course of years been found responsible for allergic manifestations. Fortunately, such studies have been made, over sufficiently representative sections of the country and are available for reference so that, with their aid, the man not primarily interested in allergy can develop a fairly satisfactory knowledge of the more important hay fever producing plants in his vicinity. Obviously it is illogical to administer ragweed extract on the Pacific Coast where ragweed is not a factor, or Russian thistle, a frequent offender of the West, in Virginia where it is of little importance, even though the patient has reacted positively thereto.

*A Pollen Calendar*—One must be well acquainted with his pollen calendar. A young lady with hay fever always beginning in August and extending to frost had been treated for three successive years with ragweed extract never with benefit. Had the doctor known that ragweed does not start to pollinate in Virginia by the tenth of August, the date on which this patient's symptoms usually begin, he would have searched elsewhere for the cause. This fact being known to us, we promptly found the cause in goldenrod and were able to give the patient adequate relief.

For best results one must also be somewhat of a field botanist. It is not enough to know what is pollenating but also how abundantly. The last pollen season in Virginia, has been the heaviest on record. During this season I took frequent rides into the country to see what was pollinating and how abundantly. While ragweed was everywhere I observed that goldenrod was almost as abundant. Acres upon acres were almost uniformly yellow with this pretty flower. Now, goldenrod is usually said not to be much of a factor, since the pollen is said not to carry more than a few hundred yards. Goldenrod pollen is not at all uncommon in my experience but we usually do not desensitize against



it because the patient can so easily avoid it. But this year with the roadside lined for mile after mile with this plant nearly any drive into the country would result in adequate exposure to produce symptoms. Furthermore we have found goldenrod pollen on our slides on the eighth floor of an office building in the center of Richmond. A child entirely satisfactorily desensitized against ragweed to which she was sensitive did have some symptoms this last season which were unexplained, until I visited her home on the outskirts of town and found that she was playing most of the day in a vacant lot next door quite overgrown with goldenrod. Her teacher also had goldenrod in the schoolroom. Coseasonal treatment was then instituted with goldenrod extract to which she was sensitive, with prompt subsidence of symptoms. It is well therefore to bear in mind that if your patient is not satisfactorily relieved, the cause for failure may be other than inadequate desensitization with the pollen or pollens with which you have been treating him.

While a very great variety of plants may be responsible for allergic symptoms, less than two dozen are responsible for fully 90 per cent of the trouble. The problem of acquiring practical experience in field botany is therefore not a very difficult one.

As a corollary to the above, we need but mention that the pollen extract used for diagnostic testing must be appropriate. One must use extracts of those hay fever producing pollens to which one knows that the particular patient will be exposed. For the man who wishes to get good results in a reasonable percentage, say 75 per cent of his patients, and who plans on sending the more difficult ones to an allergist who has a greater variety of pollens at his disposal the necessary list of extracts is not large. The list however varies in different sections of the country.

*Potent Test Extracts*—The extracts must be potent. Most of those available today through the pharmaceutical concerns are potent. With different methods of extraction the duration of the potency varies and one should therefore be acquainted with the durability of his extracts.

*Reliable Test Methods*—The methods of testing must be reliable. Both the scratch technique and the intradermal method have their advocates. Provided one has a potent extract such as a two per cent extract the scratch method will be found reliable in about 90 per cent of cases. This is true of pollens but not equally so of several of the other allergens. The factor of safety is so much greater with the cutaneous or scratch method, when we are dealing with pollens that it is the only method which can justifiably be recommended, in my opinion, to those who have not made a special study of allergy. Here again I am speaking only of the pollens.

I should mention a few points which in my experience have increased the accuracy and safety of diagnostic pollen testing.

Always perform pollen tests on the arm. If this is done the very rare untoward systemic anaphylactic reaction can be promptly controlled by the application of a tourniquet above the site of the reaction and the injection of 0.3 cc of adrenalin into the opposite arm.

Place the scratches at least one inch, preferably an inch and a half apart

Too often the scratches are placed so closely together as to result in fusion of positive reactions and confusion in reading

If the date of onset of symptoms makes you reasonably certain that a certain pollen or group of pollens is responsible, and if the skin reactions have been negative, repeat the test. False negative reactions are a distinct source of error. I have seen a frank negative in one test become a strongly positive reaction on another test made half an hour later.

If in spite of continued negative reactions you still have a reasonable doubt, the ophthalmic reaction may be applied. This method has been recommended especially by Peshkin and I have found it of distinct value. A small amount of natural dried pollen on the tip of a flat toothpick is introduced inside the lower lid. Five minutes later this is washed out with physiologic saline. The intensity of a positive reaction varies from a little redness at the inner canthus to an intense congestion of the conjunctival mucosa with some edema. The last is rare unless the skin test has been positive, and the test should not be used in the event of a positive skin reaction. The reading is made by comparison with the untreated eye, after which the reaction is promptly controlled with a drop or two of solution containing one part of adrenalin 1/1,000 and three parts of boric acid solution.

Do not desensitize with everything that gives a positive reaction. Use only those pollens which coincide on the pollen calendar with the dates of symptoms. As a rule, use only those that are wind pollinated. In my experience it is almost a rule to observe positive reactions to pollens whose presence have not caused symptoms, in addition to those which have.

#### THERAPEUTIC REQUISITES

I have discussed briefly some of the more important factors in the diagnostic study. Naturally those who will be making these studies at all frequently will refer to some of the excellent recent volumes on allergy which are now available. Many prefer to send their patients to an allergist for diagnostic survey, and these are more particularly interested in the most appropriate method of treatment after the patient's reactions have been determined and he has returned for treatment by his home physician.

*Potent Extracts and Adequate Dosage*—Among the requirements for successful treatment we must again mention the use of potent extracts. Next comes adequate dosage. A patient half desensitized is almost better not desensitized at all. I can usually obtain better results during the pollen season with a patient who comes in without having had any previous treatment than with a patient who has had half a course of treatment and then comes in with symptoms. One of the most pernicious hindrances to good results today in pollen therapy is the commercial fifteen dose treatment set. At the completion of the fifteen doses the patient is usually only about half desensitized, but since he took the whole set, the patient and usually his doctor also settle back with the assumption that adequate treatment has been given. Then, when symptoms return at the usual date, both patient and doctor conclude that there is nothing to the treatment. These sets usually provide a top dosage of three thousand units. In Virginia where

the ragweed concentration is relatively low, I always try to provide a maximum dose before the season of twenty thousand units. This is one of the most important requisites of preseasonal treatment. Some allergists in sections of the country where the ragweed count is high, provide even higher dosage.

It is unfortunate that discussion of dosage today is complicated by the rather general use of three entirely different terminologies, all of them quite arbitrary. The pollen unit is more frequently used by the commercial houses. Its advantage lies in the avoidance of the use of fractions. One pollen unit represents the active principle extracted from one millionth of a gram of dried pollen.

A second nomenclature is based upon the strength of the pollen extract in terms of nitrogen content. If all of the proteins in pollen were antigenic and if the antigen contained nothing other than protein, dosage based upon nitrogen content would be entirely satisfactory. Unfortunately, however, it has been demonstrated that the antigenic activity of a pollen extract does not parallel its nitrogen content. This appears to be due to the fact that a given pollen contains several proteins not all of which are antigenic and that other substances besides proteins, particularly polysaccharids enter into the antigenic reaction.

The third system for recording dosage is the simplest. It is probably the more frequently used by allergists. It consists merely in recording the strength of the extract as prepared. Thus, a concentrated 2 per cent extract is called 1/50. This, diluted ten times or a hundred times is designated 1/500 or 1/5,000.

Since, in your reading, you will come across the first and third classifications the more frequently, it will suffice to say that 1 c.c. of 1/50 concentration contains 20,000 pollen units, 0.1 c.c. of 1/50 contains 2,000 pollen units. The fifteen dose treatment set usually starts with a first dose of something under 20 units and ends with a top dose of 3,000 units. In the average, that is, not the extremely sensitive case, the allergist usually starts with 0.1 c.c. of 1/5,000 and increases the dose steadily to a top dose around 1 c.c. of 1/50. This top dose is obviously over six times as strong as that provided in the treatment set and the results are usually proportionately more effective.

*Control Tests*—A third desideratum for successful therapy is the development of some method of testing during the course of treatment by which we may determine when the patient has been adequately desensitized. The cutaneous reaction is not entirely satisfactory as a control test, since one may have hay fever even though the cutaneous sensitivity has been entirely abolished and, vice versa, one may be entirely relieved of hay fever by treatment even though the skin test is still positive. However, this serves as a fairly satisfactory rough method of check and one should always attempt to give sufficient treatment to render the patient negative to the cutaneous scratch reaction with a concentrated (1/50) extract.

Realizing that there is this difference between cutaneous and mucous membrane sensitivities, and that our major interest is in rendering the patient's mucous membranes insensitive to ragweed, I have attempted during the last ragweed season to develop an eye test comparable to the diagnostic ophthalmic reaction previously mentioned, and which will give us more definite information prior to the onset of the season regarding the sensitivity of the patient's mucous

membranes For this test we use saline extracts of the dried pollens in concentrations of 5 per cent, 0.5 per cent and 0.05 per cent These we designate as 1/20, 1/200 and 1/2,000 The test is applied by introducing a drop of saline extract onto the ocular conjunctiva and studying the intensity of the eye reaction. The direct application of the dry pollen as in the ophthalmologic test is not satisfactory for this purpose since it provides a concentration in the eye far in excess of any that will be met with during the pollen season While we have not completed the study of our results, I will say that three-fourths of the patients whose ocular conjunctivae are negative to 1/20 concentration will get through the season with more than 90 per cent freedom from symptoms Of those who are sensitive to 1/20 but insensitive to 1/200 two-thirds will have more than 90 per cent relief These tests were applied two or three weeks before the onset of the season, and with those who appeared from the test not to be adequately desensitized, treatment was pushed more intensively Their tests showed satisfactory improvement just prior to the onset of the season Had we not been applying this preliminary test, we would have failed to give especially intensive

TABLE I  
THERAPEUTIC OPHTHALMO REACTION

100% RELIEF WAS OBTAINED IN	
50% of cases who were negative to 1/20	
33% negative to 1/200	
8% positive to 1/200	
90% AND 100% RELIEF IN	
71.4% negative to 1/20	
66.6% negative to 1/200	
41.6% positive to 1/200	

treatment to those who apparently require more than the average, and they would not have been as satisfactorily relieved I believe that as soon as we have completed the standardization of this therapeutic eye reaction we will have available a much more valuable and direct method for controlling the progress of treatment

*Direct Control*—The doctor who has direct supervision of his patients will obtain better results on the average than the man whose patients are being treated through the intermediary of another physician This is especially true of coseasonal treatment We have developed a fairly standard method of preseasonal desensitization, but once the season has begun, the treatment varies from day to day depending upon the pollen concentration of the air This last ragweed season was unusually heavy and the coseasonal treatment that I had found satisfactory in 1930 was found to be inadequate in 1931 The much higher pollen concentration in the air required a compensatory reduction of the size of coseasonal dosage, to maintain the balance In Richmond, where we knew the amount of pollen in the air each day, we could grade our coseasonal dosage accordingly But with the patients out of town, the time required in correspondence to and from the doctor prevented this ready flexibility and the results with out of town patients were sometimes not quite as good as with those who reported to the office

*Daily Pollen Graphs*—The last of the important requisites for successful therapy is a *sine qua non*. These are the pollen graphs. No man should seriously attempt to carry on pollen therapy without knowing from day to day the pollen prevalence in the air. The procedure is very simple. An ordinary microscope slide coated on one side with white vaseline is exposed on a window ledge or on the roof, slightly protected from rain, and in the shade. Every twenty-four hours the plate is taken in and a new one replaced. The pollens are actually counted under the microscope. A mechanical stage is helpful. For standardization, the barrel of the microscope is drawn out until the diameter of the field is exactly 14 mm. This can easily be done by focusing on the squares of a hemocytometer chamber. For ragweed, a count of five successive trips across the narrower width of the slide expresses the number of pollen grains per cubic yard of air per twenty-four hours. If we wish to always express our figures per cubic yard of air, the area of the field counted will vary, depending upon the size of the pollen grains, but for practical purposes this is not necessary, since the variation is not great and our interest is usually in the relative counts of the same pollen from day to day.

With the information obtained in this way, we can plot the curve of pollen concentration for each day. In my own office this is recorded each day on a blackboard so that the patient also may study the curve (Fig 1). If, on days in which the pollen concentration has been unusually high, he has some symptoms he can understand the reason therefor and know that it was due to a temporary unusual prevalence and not to failure of treatment. Such a patient might have two or three days of hay fever or asthma during the season but he understands why, and is not dissatisfied. When asked a year later what results he obtained he will say, "I did splendidly except on two or three days, when the pollen concentration was unusually high and when I had some symptoms." A patient who has not studied such a chart and who expects to get complete relief will only remember that he did have symptoms after all. A year later he will say, "I had the treatment but I had asthma just the same." But the chief value of a pollen graph is in its control of coseasonal treatment, since as I have said, on those days on which the pollen prevalence is high, the coseasonal dose must be proportionately reduced.

Some years one will feel that he is obtaining consistently good results from his pollen therapy, and the next year it may be that with the same treatment the results are disappointing. The pollen graph will show however that on different years the pollen concentration varies decidedly (Figs 2 and 3). Variation in results was due not to the treatment but to the pollen concentration. Meteorologic conditions are chiefly responsible for the varying pollen concentration. In 1930 Virginia was hard hit by the drought. There was no rain in August until the twenty-eighth when we had a heavy rainfall of 1.8 inches. There was as a consequence a ten day delay in the onset of the pollen season and hay fever victims who had had no treatment were jubilant with the thought that at last they were free of the disease. But there is an old saying, "nothing is certain in the future save death, taxes and hay fever." Within three days after the rain of August 28, ragweed commenced to bloom and, making up for lost

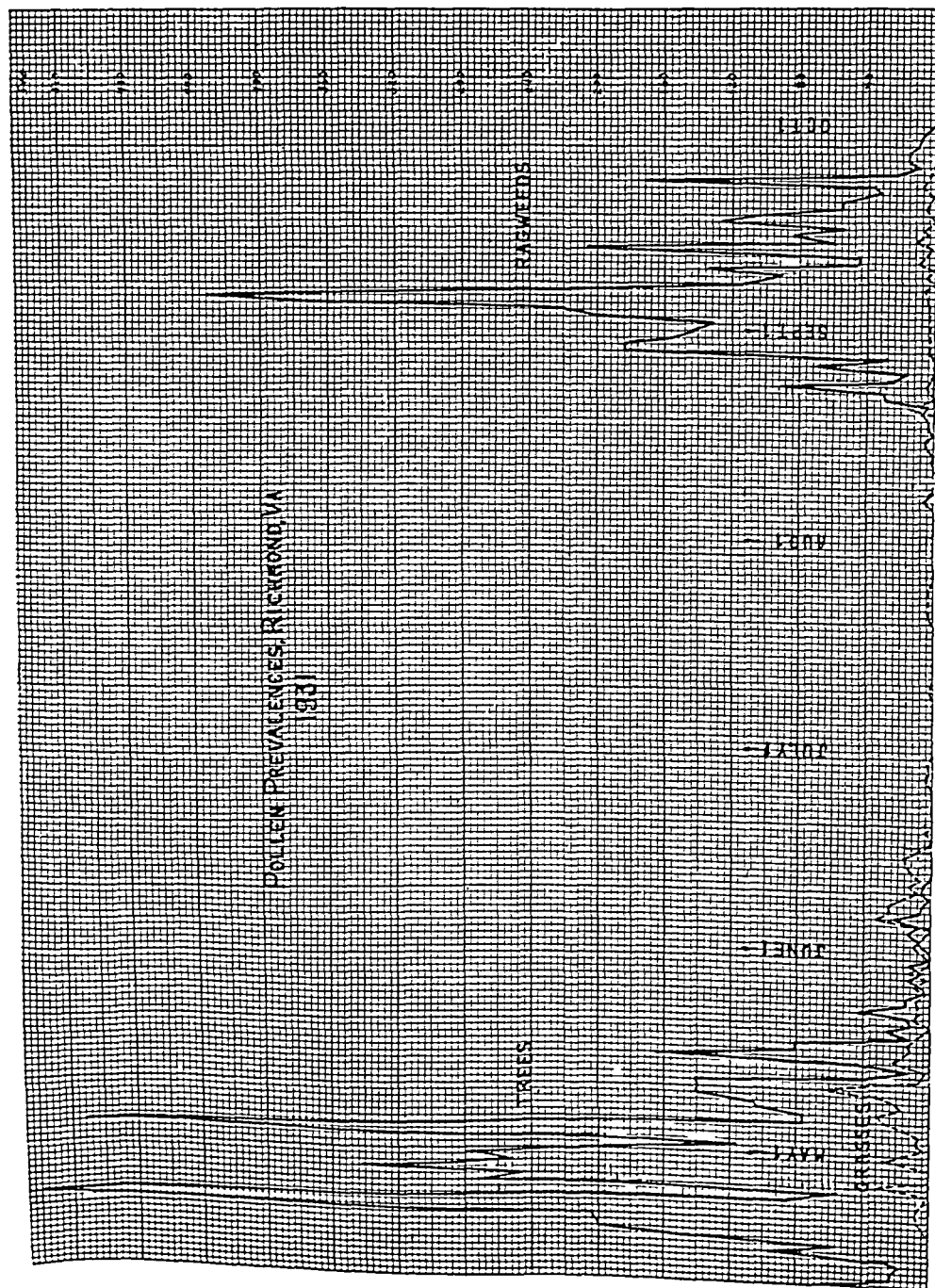
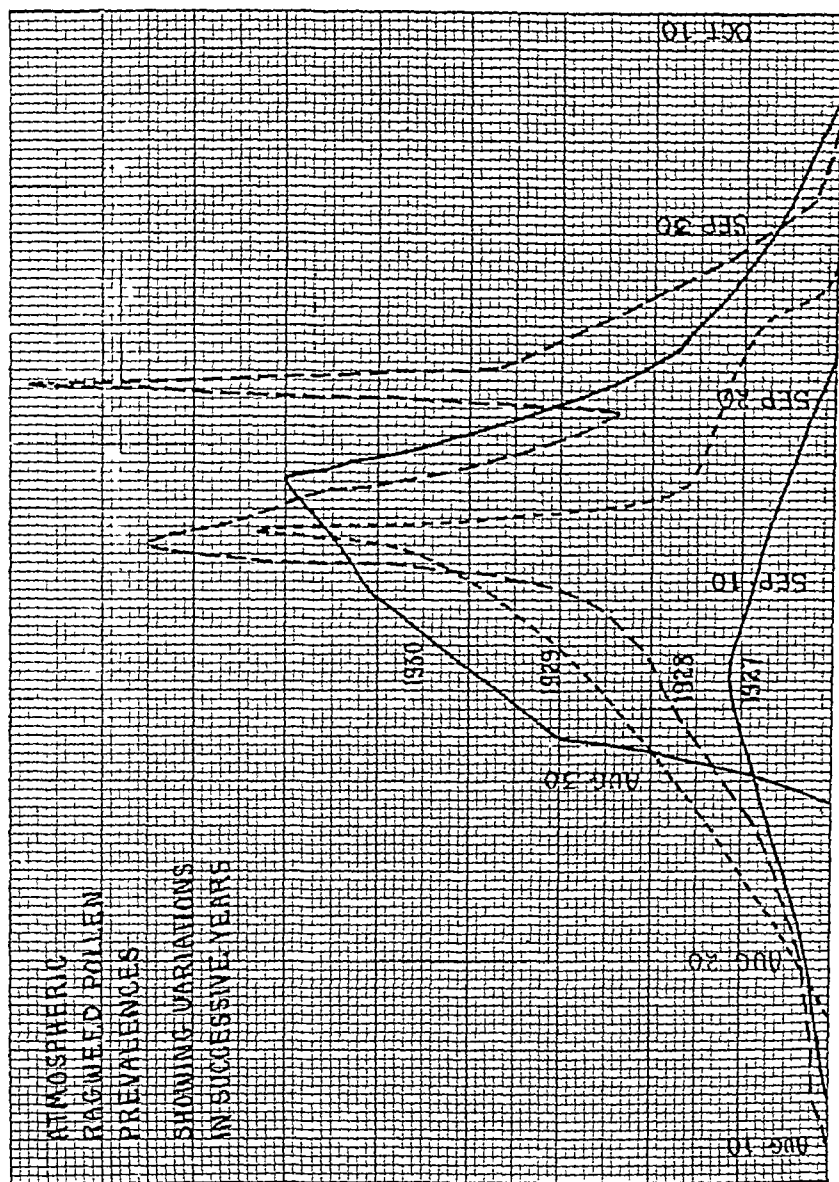


Fig 1—Pollen prevalences in Richmond in 1931. The tree curve represents all varieties of tree pollens. The ragweed curve there-  
fore represents the heaviest individual pollen prevalence for year.

time, shed in unusual abundance, so that after the first of September we had a pollen concentration heavier than the average (Fig 2)

The 1931 curves are most interesting (Fig 3) We had drawn a probable curve before the onset of the season representing the mean of the preceding four



1 line, 2 — Variations from year to year of that year After pollen distribution once started it continued, heavy average  
The delay in the start of the 1931 curve was due to the drought

years To our surprise ragweed first appeared on pollen plates a week earlier than usual, and the concentration through the season ranged from three to seven times heavier than any other season since we first began keeping records This unusually high prevalence of 1931 appears to have been rather nation-wide As far as Virginia is concerned there appear to have been three causes First, as we have just seen, in 1930, when it finally started there was an unusually heavy

distribution of pollen, and therefore an unusually heavy fertilization of seed. Second, July in Virginia was unusually hot, the average high temperature being 80, two degrees hotter than usual. But the rainfall was normal, 4.5 inches. Therefore, ragweed like all other vegetation grew luxuriously in July. Third,

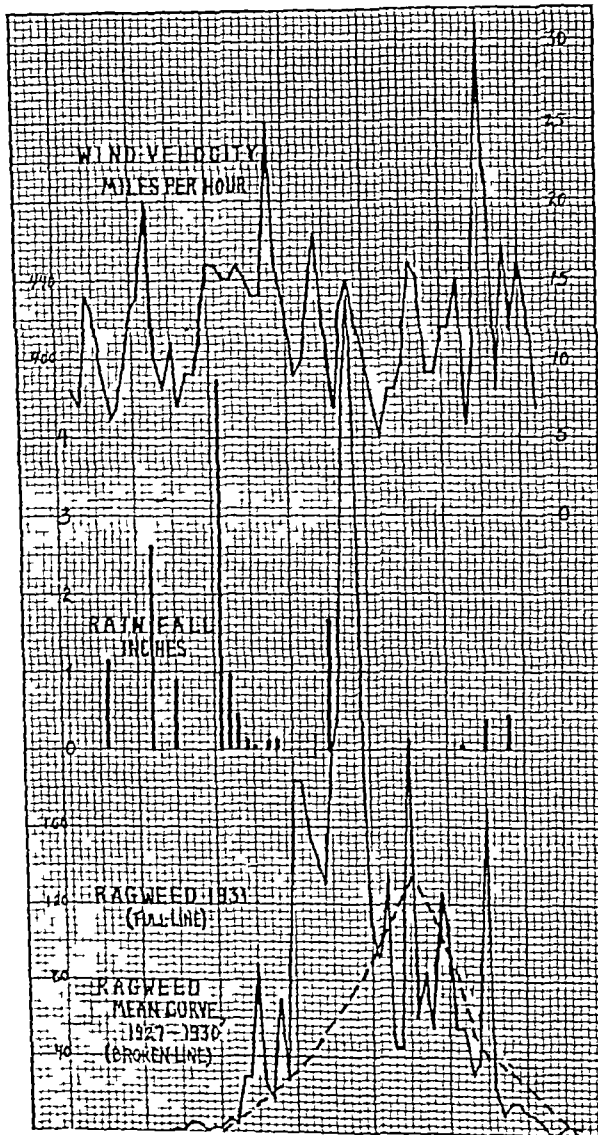


Fig. 3—Showing the effect of high winds in producing an unusually high pollen prevalence in the first half of the 1931 season. Also towards the end of the season. Dotted line represents the mean curve for the preceding four years.

In August we had the heaviest rainfall (11.4 inches) that has been recorded since the weather bureau was started in 1872. These three factors, wide distribution of seed, abundant heat and sunlight and plenty of water combined to cause the unusual prevalence of this year. Add to this several days of heavy wind early in September, which stirred large amounts of pollen into the air. Very early in



this season we discovered that the coseasonal maintenance dose of pollen extract which worked beautifully last year was causing reactions. The answer was at once apparent in the higher atmospheric concentration and we promptly cut the size of our coseasonal dose. Without the use of our pollen graph we would not have cut the dose, patients would have had frequent reactions, and the results this year would have been poor. So, it is obvious that pollen graphs are essential for best results.

The graph for one locality may not be usable in another. The wind that precedes a storm always stirs large quantities of pollen into the air and local showers may therefore cause curves to vary in different localities. Ragweed pollinates at different times on the mountain and in the valley a mile or so away. The curves also vary for different sections of the country. Durham has shown that pollination begins and ends earlier in the north and in the central states than in the south. The average concentration likewise varies. The average prevalence along the Atlantic Coast is always lower than that of the Mississippi Basin.

With potent therapeutic extracts, with knowledge of the requisite top dose for preseasonal treatment, with appropriate tests for control of the rapidity and thoroughness of desensitization and with knowledge of the pollen concentration of the air during the season, one is properly equipped for therapy.

#### THERAPEUTIC METHODS

There are in general four methods of treatment, preseasonal, coseasonal, perennial and rush.

*Rush Inoculation* which is being cautiously studied by Freeman in London has not and probably will not come into general use in this country. It consists in hospitalizing the patient and giving inoculations at intervals of hours instead of days, building up to top dose within a matter of a few days. The possibility of anaphylactic reaction following the giving of such tremendous doses in a short period is great and the patient must therefore be hospitalized and under closest supervision to control reactions when they do arise. In England where pollenosis is due to the less toxic grasses, this may be possible but in this country where the chief offender is the much more toxic ragweed, it is a method of treatment that must be approached with the utmost caution.

*Preseasonal Treatment*, with injections once or twice weekly has been the common method of desensitization. When properly given this will furnish adequate relief in fully 75 per cent of patients. The most important factor in proper administration is the reaching of an adequate maximum dose prior to the onset of the season. In some localities 0.2 c.c. of 1/50 concentration or 4,000 pollen units will suffice. A safer minimum top dose is 0.5 c.c. or 10,000 pollen units. One cubic centimeter of 1/50 or 20,000 pollen units will protect in almost any locality in the United States. With some patients this high dosage must be approached with caution. The extremely sensitive may experience anaphylactic reaction when approaching this top dose. In this case treatment must be increased more slowly. I have had but one patient who has not eventually tolerated a dose of 20,000 units without trouble. And if this dose can be reached prior to the season, I feel confident of satisfactory if not complete relief irrespective of whether or not control tests have been done.

One occasionally finds an extremely sensitive patient who cannot safely receive the initial dose of 0.05 cc of 1/5,000 or 10 pollen units. The most sensitive that I have encountered had to receive an initial dose of 0.1 cc of 1/2,000,000. Therefore, in order to safeguard against this occasional extremely sensitive individual it is best before starting treatment to do scratch skin tests with the dilutions of pollen extract, 1/50, 1/500 and 1/5,000 and 1/50,000, and to start treatment with that concentration which just fails to give a positive skin reaction.

There is a rapid preseasonal method applicable to those who present themselves for treatment too late for one to attain the top dose by gradual increases at weekly or semi-weekly intervals. Suppose a patient reports for desensitization just two weeks prior to the onset of the season. Here, the treatment may be given daily or even twice daily but the doctor must be more alert for anaphylactic reactions and must be prepared to control them.

*Coseasonal Treatment*—Originally it was thought that desensitization treatment could be given safely only before the season and that, once the season had commenced, treatment must be discontinued. Otherwise there was great danger of anaphylactic reaction. For the patient to whom preseasonal treatment did not give adequate relief there was nothing more to be done.

In 1923 the speaker first described the treatment of a series of patients who presented themselves for the first time during the season and without previous therapy. Since then I have varied my treatment, obtaining good results some years and less good in others. In two seasons I attempted rapid coseasonal desensitization, with inoculations daily or twice daily or every other day, running the dose up rapidly to the usual top dose. The last year I have reverted to my original method and have conclusively demonstrated its superiority. The general principles may be outlined as follows:

The principle of coseasonal desensitization is completely different from that of preseasonal treatment. In the latter the whole aim is to reach a high top or maximum dose. According to the theory of immunity this results in either the exhaustion of fixed antibodies attached to the cells or the compensatory production of so many free antibodies in the blood stream that the antigen as it enters the respiratory passages is neutralized by the free antibodies before it has an opportunity to combine with the fixed cellular antibodies. A high preseasonal dose is the desideratum.

In coseasonal treatment no such attempt is to be made and if it is made it results in failure. Frequent small dosage is the secret to success in coseasonal treatment. One starts in with a small dose, say 10 or 20 pollen units or 0.1 cc of 1/5,000 and gives daily inoculations. Improvement is usually almost instantaneous. I have seen it occur within thirty minutes. The dose is increased by 20 units each day until satisfactory relief results, and rarely is it necessary to exceed 80 units daily for the obtaining of 75 to 100 per cent relief. As soon as this is accomplished the interval between doses may be increased by one or two days. We then attempt to steadily increase the interval up to once or twice weekly. If symptoms return doses are again given daily, and if they persist in spite of this the dose is cautiously increased.

In coseasonal therapy keep the dose small to obtain maximum benefit, and give it as frequently as necessary to maintain the benefit. If you continue to push the dose up in spite of relief, you will reach a stage where the combined dose from your inoculation and from the inhalation of pollen is too great, and then the patient's symptoms will become worse instead of better.

*Perennial Treatment*—The third method perennial treatment, follows the completion of either preseasonal or coseasonal therapy. It is no small task to adequately desensitize a patient prior to the season. It seems poor logic, directly the season is over, to discard all of the advantage gained, allow the patient to again develop his sensitivity, and to have to repeat the entire process a year later. In administering perennial treatment the injections are continued slightly below the maximum dose, being given every two weeks throughout the year. Prior to the next season the task of rapid desensitization need not be repeated. It will only be necessary to step up the dose slightly, to the usual maximum. For example, with a top dose of 20,000 units just prior to the season we will drop down as soon as the season commences to a maintenance dose of 2,000 or 3,000 units every two weeks through the season and on through the year. A short time before the next season this will again be stepped up, in the course of four or five treatments to the usual preseasonal maximum of 20,000 units or 1 c.c. of 1/50 concentration.

The advantages of perennial treatment which was first described by Stewart and by Brown are (a) better results (b) avoidance of the inconvenience and labor to both patient and doctor of intensive preseasonal desensitization, (c) diminished tendency to anaphylactic reactions, (d) distribution of the doctor's work throughout the year, (e) the possibility of permanent immunity after three or more years of such treatment, (f) treatment may be started at any time of year.

In both preseasonal and perennial treatment it is well to drop from the maximum to the maintenance dose immediately with the onset of the season.

*Anaphylactic Reactions*—A word about prevention and control of anaphylactic reactions. This is the *bête noire* of allergic therapy. And yet it is easily avoided and almost invariably promptly controllable. Except in the most fulminating reaction, the symptoms consist in urticaria, violent sneezing, or asthma coming on within a variable period after inoculation. A reaction of any great severity will occur within the first half hour or forty-five minutes. Reactions may occur after this but are usually easily controlled with ephedrin. If therefore the patient remains in the office thirty to forty-five minutes he can safely leave, provided he understands the symptoms of reaction and that he is to take ephedrin in the event any of these symptoms appear. He should understand that he is to take his ephedrin capsule and report back at once to the office.

Reactions at any time are usually promptly controlled by the methods outlined by Duke and Insley. Immediately upon the appearance of symptoms a tourniquet is placed upon the arm above the site of inoculation and 0.3 c.c. of adrenalin is introduced into the other arm. Symptoms are usually promptly relieved and the patient rapidly experiences adrenaal symptoms. The tourniquet is loosened and tightened at intervals over a period of half an hour to an hour.

or two, until it can finally be left off permanently without return of symptoms. All of those who attempt desensitization therapy should read the articles by Duke and Insley in the original.

As a precautionary measure, when any treatments are given during the season, we always administer 0.3 cc of a mixture of equal parts of 1/1,000 adrenalin and 3 per cent ephedrin in the same syringe with the allergen extract.

## RESULTS

The steady improvement in allergic therapy is exemplified in the report of Cooke, Vander Veer and Spain in which they have compared their results from year to year. In 1915 they obtained excellent results in 4 per cent. Eleven years later they obtained excellent results in 39 per cent. Their good results for 1915 total 47 per cent and in 1926 50 per cent. Their poor results in 1915 total 31 per cent and in 1926 11 per cent. No improvement was obtained in 15 per cent in 1915 as contrasted with only 3 per cent in 1926. Gay has concluded that on the average, desensitization therapy reduces the period of seasonal discomfort from a month and a half to a week and a half.

TABLE II  
RESULTS 1931

RELIEF (%)	PERENNIAL		PRESEASONAL		COSEASONAL	
	No.	%	No.	%	No.	%
100	12	46.3	1	7.6	6	40.0
90	6	23.1	5	38.5	3	20.0
75	6	23.1	5	38.5	4	26.7
50	2	7.5	2	15.4	2	13.3
Satis- factory 75-100%		92.5		84.6		86.7

TABLE III  
RESULTS 1930 AND 1931

METHOD	SATISFACTORY RESULTS (75-100% relief)
Coseasonal 30 cases	73.3%
Preseasonal 34 cases	79.4%
Perennial 47 cases	93.6%

All allergists have had similar experience. Comparable improvement has been achieved in each of the three common methods of treatment. In my own experience the four outstanding recent advances have been (a) increase in the maximum preseasonal dose, (b) the development of control tests, (c) frequent and small coseasonal dosage and (d) perennial treatment. Undoubtedly perennial treatment has in my experience been productive of best results. Up until this year I have felt that preseasonal treatment was superior to coseasonal. In 1930 100 per cent of our patients obtained satisfactory relief following perennial desensitization. Seventy-one per cent obtained comparable relief from presea-

sonal treatment Sixty per cent were adequately relieved from coseasonal treatment This year, however with the establishment of the principle of small dosage, our coseasonal results have been as good as our preseasonal Either method, should, however probably be followed by perennial therapy

#### CONCLUSION

Twenty years have now elapsed since desensitization to pollens was first undertaken by Noon and Freeman Results, at first mediocre, have been gradually improved year by year until at last we have reached that stage where, provided the experience and principles now available are properly applied, one may proceed with treatment, secure in the knowledge that his patient will receive with very rare exceptions from 75 to 100 per cent relief from symptoms This is truly a remarkable achievement and represents the results of collaborative effort of a large group of earnest students of allergy

We have almost reached that stage where we can modify the ancient dictum, in saying "nothing is certain in the future except death and taxes"

808 PROFESSIONAL BUILDING

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### OBSERVATIONS ON THE ACCURACY OF THE RABBIT OVULATION TEST FOR PREGNANCY\*†

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CHIEF among the requisites of a good clinical test we must list accuracy, simplicity, and rapidity Thus in considering the various hormone tests for pregnancy, we find that Friedman's modification of the Aschheim-Zondek test<sup>1</sup> is apparently the most rapid, since results are obtained within forty-eight hours It is also extremely simple, many investigators using but one injection of urine into a rabbit In addition, a macroscopic examination of the ovaries for corpora hemorrhagica suffices, thus obviating the necessity of histologic preparations The accuracy of this method, however, has not yet been fully established Out of over 1000 tests reported in the literature we find approximately ten errors, resulting in an accuracy of over 99 per cent Even at that some of these errors were undoubtedly due to various technical mistakes This is superior to the accuracy of from 96 per cent to 98 per cent for the Aschheim-Zondek test, as reported by White and Severance<sup>2</sup>

Our investigation was begun in June, 1931, with the idea of using the rabbit ovulation test only in those cases in which the diagnosis of a possible pregnancy was imperative, but doubtful, according to the usual means of examination Thus we believed, might constitute a more rigorous and valuable test of the method,

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than would the indiscriminate application of it to cases easily diagnosed by other procedures

Table I shows the different techniques and results of various investigators

TABLE I

	AMOUNT INJECTED	TIME	NO CASES	ERRORS
1 Friedman and Lapham <sup>2</sup>	4 cc tid for 2 days	48 hr	92	0
2 Schneider <sup>12, 13</sup>	5-7 cc	24-30 hr	200	2
3 Reinhart and Scott <sup>14, 15</sup>	5 cc	24 hr	150	2
4 Martins <sup>6</sup>	5 cc	48 hr	100	0
5 Magath and Randall <sup>7</sup>	7-15 cc	30 hr	85	1
6 Bradford and Todd <sup>8</sup>	10-12 cc	24 hr	22	0
7 Davis and Walker <sup>9</sup>	10 cc	24 hr	65	2
8 Beasley <sup>10</sup>	?	?	52	0
9 Wilson and Corner <sup>11</sup>	5 cc	16 hr	196	1
10 White and Severance <sup>2</sup>	10 cc	48 hr	40	1
11 Ware and Main	10 cc	48 hr	100	1
(Results here reported)			1102	10

The age and weight of the rabbits are of no particular consequence so long as the animal is mature, since some breeds are much larger than others, and may mature at different ages. The rabbits we use are virgin female chinchillas, five months of age. They are obtained from a breeding farm, so that all are of the same strain, age, and weight, and have been completely isolated from males for several months. Of late we have kept these rabbits in individual cages a week prior to use, in order to prevent possible formation of corpora hemorrhagica through mechanical stimulation of the genitalia. The urine injected was the first specimen voided in the morning. Samples collected from pregnant patients at other times during the day often give weak positives, probably due to a lack of concentration of the hormones. No aseptic precautions were taken, but unless the urine was to be used within a few hours, it was kept on ice. Nevertheless, several specimens which had been kept at room temperature for several days still produced good results.

During the first 20 tests we used two injections of urine of 10 cc each, into the marginal ear vein. The second injection was made twenty-four hours after the first, and the rabbit killed forty-eight hours following the first injection. This method may be advisable in cases of very early pregnancy where the hormones are not concentrated, but in order to simplify the test as much as possible, for the rest of the investigation we used but one injection of 10 cc, killing the animal in forty-eight hours. This happens to be exactly the same method as used by White and Severance.<sup>2</sup> Many investigators examine the test animal at the end of twenty-four hours or less. In our experience however, this has not proved long enough. Occasionally wishing to obtain a result quickly, we have injected two rabbits with the one specimen of urine, killing one rabbit in twenty-four hours, so that should the results appear doubtful, we could kill the second animal in forty-eight hours. In two cases among these twenty-four-hour rabbits gave doubtful findings, the follicles being very large but quite clear. Both forty-eight hour

rabbits gave the typical results of a positive test. This makes us feel that twenty-four hours is not always sufficient time, in spite of the excellent results of Wilson and Corner,<sup>11</sup> who allow but sixteen hours to elapse before examination. Occasionally we have allowed seventy-two hours to elapse, without any detriment to the results.

Besides the many corpora hemorrhagica found in the ovaries of rabbits injected with pregnant urine, we have noticed that the uterus is also much enlarged, and generally very hyperemic, in comparison to the small white uterus usually present in negative cases. This difference is so striking that in the majority of the cases a diagnosis may be made when the uterus is exposed, and before examining the ovaries. This sign has proved of value in three cases of apparent spontaneous formation of corpora hemorrhagica of rabbits in which urine from non-pregnant patients had been injected. As indicated by Wilson and Corner,<sup>11</sup> there is not much difficulty in the differentiation of these false positives by the fact that the corpora hemorrhagica are a dull red and the follicles are rounded rather than conical. In these three cases, there were only from one to three corpora hemorrhagica present, and the uterus was small and pale. Occasionally, but not often, nonpregnant urine will cause enlargement of the uterus, or pregnant urine may not produce uterine enlargement. This uterine hypertrophy is more noticeable in virgin females than in those which have had young. Another advantage in using virgin rabbits is that their ovaries are small and clean, no traces of degenerating corpora lutea being present, so that results are unmistakable.

The only error we have found was a negative result following injection of urine from a patient found to be pregnant at operation. Urine collected a few hours following operation gave an excellent positive reading, as did specimens for two days following the hysterectomy. Since the first urine sample had been collected according to the ordinary ward routine, there is of course a possibility that an interchange with a specimen from another patient had occurred. It seems highly improbable that a specimen collected prior to operation should give a negative test, since those following hysterectomy gave positive readings.

A comparatively large number of injected rabbits died, possibly because of the so-called toxic urines. Out of over 170 rabbits injected, fifteen died within ten minutes to twenty-four hours following injection. In no case was this due to cloudy or alkaline urine, for all cloudy specimens were filtered and although we do not routinely test the acidity of the urine, this was done in every case following the death of the rabbit. In none of these was an alkaline urine found. Five of these deaths were caused by urine from a case of hydatidiform mole and four from a patient with chronic nephritis and following a therapeutic abortion. The other deaths were scattered, but occurred during the extremely hot weather of the past summer, and may have been in part due to the high temperature. We have not tried the detoxification of such urines by extraction with ether, as recommended by Zondek.<sup>12</sup> Martins<sup>6</sup> believes that such a procedure actually increases the sensitivity of the test.

The explanation of the formation of corpora hemorrhagica in the ovaries, following injection of urine from a pregnant patient, is that these results are now believed to be due to the production of hormones by the placental tissue, and their subsequent excretion in the urine. Among these hormones are found two which

closely simulate those produced by the anterior lobe of the pituitary. These cause maturation of the follicles, ovulation, formation of corpora hemorrhagica and later corpora lutea. Indirect proof that the placenta may serve as a source of hormones, has been offered by several investigators. Collip<sup>13</sup> has demonstrated that human placental tissue contains large quantities of hormones. Others<sup>14</sup> have found that following castration of pregnant women, the urine at term contained at least the female sex hormone. This apparently proves that other tissues, presumably placental, were able to secrete this hormone in the complete absence of the ovaries. Again, Wilson and Corner<sup>11</sup> found that in cases of incomplete abortion, positive results with the rabbit test are obtained so long as living placental tissue remains. Both the hydatidiform mole and chorionic epithelioma also apparently produce these hormones, since positive results with ovulation tests are obtained in these cases. In fact, Ehrhardt<sup>15</sup> reports that the diagnosis of hydatidiform mole may be made because of the extremely large amounts of hormones excreted. This evidence, although indirect, leads one to believe that the placenta actively forms these substances rather than merely serving as a storage place for them.

Snyder and Wislocki<sup>16</sup> report that urine from pregnant animals such as the monkey, rat, cat, and dog, do not give a positive rabbit ovulation test.

The excretion of these hormones in the urine may be due to the huge quantities present in the blood. Ehrhardt<sup>17</sup> transfused nonpregnant women with 500 c c of whole blood obtained from pregnant women and demonstrated the presence of hormones in the recipients' urine with the Aschheim-Zondek test.

#### CLINICAL REPORT

We have tested specimens from 150 patients, and have followed 100 of these patients until the presence, or absence of pregnancy could be proved. A total of 135 specimens of urine have been tested on these completed cases.

One hundred patients were examined because pregnancy was questionable. This group may be subdivided into two groups:

a. Sixty three patients who submitted 87 specimens and had a similar number of tests completed. All were positive.

b. Thirty seven patients who submitted 48 specimens and had a similar number of tests completed. All were negative.

##### Analysis of positive group

There were 4 cases of early pregnancy giving positive results.

The first was examined thirty days after her last menstrual period or six days after the patient expected to menstruate.

The second was examined thirty five days after her last menstrual period or seven days after she expected to menstruate.

The third was examined forty two days after her last menstrual period or twelve days after she expected to menstruate.

The fourth was examined forty days after her last menstrual period or twelve days after she expected to menstruate.

One case, Mrs. O. M., had missed three periods. She was diagnosed as a case of hydatidiform mole, and gave a positive test. Three days later she passed a part of the mole and was curetted lightly. Following the curettage fourteen tests were made over a period of two months. Five rabbits died in from ten minutes to twenty four hours after injection and cannot be reported as test animals. Five rabbits gave positive tests before operation. A hysterectomy was done on this patient two months after she passed the mole, and specimens



tested daily. The test was positive on 1 specimen collected twenty four hours after operation and negative on 1 specimen of urine collected forty eight hours after the hysterectomy (Table II)

TABLE II

MRS O M, LAST MENSTRUAL PERIOD MAY 19, 1931 ADMITTED TO MEMORIAL HOSPITAL,  
AUGUST 4, 1931

DATE	RABBIT TESTS	RESULTS
Aug 5	Rabbit injected	Positive
Aug 11	Passed mole	—
Aug 12	Curetting	—
Aug 13	Rabbit injected	Positive
Aug 14	Rabbit injected	Positive
Aug 17	Rabbit injected	Died in 24 hours
Aug 20	Rabbit injected	Died in 24 hours
Aug 27	Rabbit injected	Died in 24 hours
Sept 2	Rabbit injected	Died in 24 hours
Sept 9	Rabbit injected	Died in 24 hours
Sept 9	Curetting	—
Sept 18	Rabbit injected	Positive
Oct 4	Rabbit injected	Positive
Oct 6	Rabbit injected	Positive
Oct 10	Hysterectomy	—
Oct. 11	Rabbit injected	Positive
Oct 12	Rabbit injected	Negative
Oct 13	Rabbit injected	Negative
Oct 14	Rabbit injected	Negative

Three patients with tubal pregnancies were tested and all gave positive tests. The earliest test was made six days after the menstrual period was due. These three patients were operated upon and the evidence of tubal pregnancy was positive. Two of these cases were proved to be tubal pregnancies by microscopic examination of the tubes. One case at the time of operation was clinically a ruptured tubal pregnancy but was not studied further.

Two patients with multiple pregnancies gave positive tests before delivery.

Two patients, one with hypertension and the other with preeclamptic toxemia also gave positive readings before delivery. In one of these cases the pregnancy was interrupted at the second month, by abdominal hysterotomy under spinal anesthesia, and every rabbit injected after the abortion died almost immediately, even with a decreased dose of urine. Evidently the toxicity of the urine increased decidedly after termination of the pregnancy. The remaining cases in this group were later proved to be normal pregnancies.

The group which gave negative tests was composed of 37 patients and may be divided as follows:

- 2 patients with ovarian cysts
- 3 patients with tuboovarian abscesses
- 1 patient with tubercular peritonitis
- 2 patients with fibroids of the uterus
- 1 patient with a missed abortion
- 2 patients forty one years old later confirmed as cases of delayed menstruation, probably because of the menopause

One patient, reported as a missed abortion, gave a negative test. She had missed four menstrual periods and had been clinically diagnosed as pregnant.

During the six weeks prior to the time her urine was tested her uterus had not enlarged. Two days after the specimen of urine was tested this patient passed a macerated placenta and fetus. The uterus was curetted but no normal healthy placental tissue was found.

This case further substantiates the finding of Wilson and Corner<sup>11</sup> who reported two cases of missed abortions. Positive tests were obtained in both during early pregnancy, and negative ovulation tests were produced by both cases before the uteri were emptied. A few days later macerated placentas were obtained in each case. Reinhardt and Scott<sup>5</sup> also reported one case of missed abortion which gave a negative test. Autopsy on this case revealed a detached and macerated placenta.

Two patients thought to be pregnant gave negative tests, and were proved at the time of operation to have uterine fibroids with no evidence of pregnancy. These cases give further evidence that uterine fibroids do not give a positive test, and help to substantiate the conclusions of Davis and Walker<sup>9</sup> that the positive test obtained by them from a patient with uterine fibroids was a technical error.

The remaining patients in this group were complete abortions.

Five patients formerly reported in the positive group deserve further mention as they were tested after delivery. The earliest negative reaction was obtained twenty-four hours after delivery, and the latest seventy-two hours after delivery. These results correspond with the findings of Wilson and Corner<sup>11</sup> who made daily tests on 16 patients after delivery.

#### SUMMARY

We have tested 150 patients for suspected pregnancy with the rabbit ovulation test, and later established the diagnosis clinically in 100 of these. This test has proved to be accurate with but one possible exception. The number of cases now reported in the literature is over 1000, with an accuracy of over 99 per cent.

We have found the rabbit ovulation test to be of particular advantage in the diagnosis of hydatidiform mole, missed abortion, ectopic pregnancies, uterine fibroids, menopause, and tuboovarian abscesses. The earliest pregnancy giving a positive result was six days after the menstrual period was due. Following delivery a negative result was obtained in from twenty-four to seventy-two hours.

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## SEDIMENTATION RATES OF THE ERYTHROCYTES IN GENERAL DISEASE\*

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SINCE the initial publication by Robin Fahraeus on the "Suspension Stability of the Blood," some ten years ago, numerous other articles have appeared and the determination of the sedimentation rate of the erythrocytes has become one of the more usual laboratory tests. The simplicity of this test, and the striking clean-cut positiveness, or negativeness, of the technical results, making it a test easily read and recorded, with a tendency to definitely fall into upper or lower registers, make it one of the useful tests in the hands of the clinical pathologist.

The clinical interpretation of the results is another matter. All the information on this is not yet at hand. Scattered reports on special diseases have been made but the test in general diagnosis has not yet been completely evaluated.

The test has been used in tuberculosis and is of considerable value when correlated with other blood findings. In acute appendicitis, a disease where early diagnosis is essential, the test does not add especially to our diagnostic acumen. Adams Ray<sup>1</sup> showed that it was not of great value in the diagnosis of cancer and our results substantiate his work. In the early cases the rate may be normal or slightly increased, and in the late cases it may be only slightly pathologic. The accompanying circumstances, the presence or absence of infection or ulceration, the extent of tissue involved, and the situation of the tumor, apparently have much more to do with the sedimentation velocity than the mere presence or absence of a malignant process.

The estimation of this rate, technically, is accomplished by one of two methods, using a standard time and measuring the distance of the settling cells, or by using a standard distance and noting the variable time. The first is the method of Fahraeus and Westergren. The second is generally called that of Linzenmeier. In this series, we have preferred the second method although they have been checked against each other and are perfectly comparable.<sup>2</sup>

Briefly the technic is as follows: a small Linzenmeier test tube, graduated at 1 c.c. and being about 50 mm. long, graduated as well at 6, 12, 18, and 24 mm. from

\*From the Mary McClellan Hospital.  
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the 1 c c mark is used Two-tenths cubic centimeter of a fresh 5 per cent solution of sodium citrate is drawn into a sterile "insulin" syringe, and 0.8 c c of blood is added directly from the patient The resulting 1 c c is placed directly in the test tube, without undue agitation, and the time noted The number of minutes required for the red cells to settle down to the 18 mm mark and leave the clear

TABLE I

DIAGNOSIS	NO OF CASES	SEDIMENTATION TIME IN MINUTES		
		HIGH	LOW	AVERAGE
Normals				
Male	5	302	130	254
Female	8	414	120	226
Neurasthenia	6	361	125	224
Acute rheumatic fever	5	39	5	22
Atrophic arthritis	16	33	9	21.8
Osteoarthritis	5	49	40	44.5
Renal or ureteral calculi	4	377	109	262.2
Cystitis	17	70	13	27.5
Pyelitis	3	18	8	13.1
Perniciou anemia	4	39	11	28.7
Pelvic abscess	10	50	8	24.1
Localized infections	19	140	12	40.2
Septicemia	7	33	8	18.6
Phlebitis	3	55	9	24.3
Tuberculosis, active	10	31	6	16.1
Tuberculosis, healed	3	316	125	194.2
Lobar pneumonia	7	81	15	35.3
Bronchopneumonia	6	78	11	35.7
Acute bronchitis	5	82	14	45
Chronic bronchitis	3	83	17	47.3
Influenza	5	197	29	66.2
Diabetes	11	340	51	186.2
Diabetes with gangrene	4	47	27	35.5
Chronic cardiac valvular disease	13	434	60	198
Acute cardiac disease	7	187	21	48.7
Appendicitis acute				
Preoperative	1	129	129	129
Postoperative	9	260	24	59.2
Appendicitis chronic				
Preoperative	4	205	40	116.5
Postoperative	9	260	5	110
Tumors				
Nonmalignant	9	148	11	62
Malignant	26	221	7	52.2
Pregnancy				
Perniciou vomiting	2			
Incomplete abortion	3	42	19	30.5
Premature delivery	1	113	63	89
Toxemia, eclampsia	3	31	31	31
Normal postpartum	4	51	35	42
Hernia, pre and postoperative	15	136	98	115.7
Fractures	31	310	26	121.2
Cholecystitis, chronic	3	158	8	66.3
Cholecystitis acute	3	230	66	139
Catarrhal jaundice	2	32	17	24.7
Cirrhosis	2	310	70	190
Arteriosclerosis	8	22	11	16.5
Hypertrophy of prostate	9	278	20	116
Total	348	200	13	108

plasma above is called the sedimentation time. As is shown in Table I, this will vary from a few minutes, in the more acute cases, to several hours in the normal individual.

This condition is thought to be<sup>3</sup> one of the fundamental responses of the body to disease and comparable to fever or leucocytosis. The mechanism is probably a relative increase in the globulin and fibrinogen, with a decrease in albumin in the serum. This changes the surface tension and the viscosity of the serum though the physical-chemical relationship is doubtlessly complex and may involve other factors.

Clinically, an increasing sedimentation time means that the patient is improving, and conversely a diminishing sedimentation time means that the patient is getting worse. A short time (a rapid rate) indicates the presence of some severe inflammatory condition, a longer one the absence of inflammation.

To gain an adequate idea of the value of this test, we ran a series on 348 of our patients, with the results shown in Table I and Figs. 1, 2, and 3. These were gen-

Sedimentation Rate in Normals

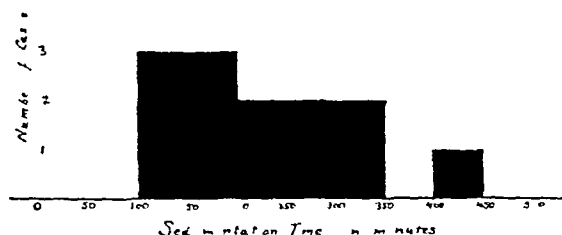


FIG. 1

eral hospital patients, both medical and surgical, and include a wide variety of clinical conditions.

First on the list is a group of 13 normals—nurses, technicians and doctors, or those hospital patients admitted for observation and showing no symptoms or signs of disease. These were all over two hours, extended up to six hours with an average of around four. In our experience this has been quite uniform, and any rate under two hours, or especially under one hour, leads to further study to ascertain the type and extent of organic disease.

Then we studied an interesting group of patients with joint disease. Five cases of acute rheumatic fever showed an average sedimentation time of 22 minutes. This agrees with the work of Einstene<sup>4</sup> who found that the sedimentation rate in acute rheumatic fever showed an even greater relative increase than the leucocyte count, and that this index remained positive several days to several weeks after the white count had become normal. The test became positive very quickly after the individual clinically showed swollen joints and was positive even in cases where the white blood count was low.

A group of 16 cases, in which the clinical diagnosis of atrophic arthritis had been made, had an average time of 21.8 minutes varying in the relatively narrow limits of 9 to 33 minutes. Five osteoarthritis varied from 40 to 49 minutes, with

an average of 44.5. This agrees with the work of Dawson, Sia and Boots<sup>5</sup> from the Presbyterian Hospital in New York, in which they found that the sedimentation rate of the red cells was parallel to the severity and extent of the process, though in cases of the so-called osteoarthritis the sedimentation rate, while as a rule, is increased, rarely attains values obtained in the other type of the disease. They report a list of 196 cases.

A third group of 6 in which the clinical diagnosis of neurasthenia was made showed times varying from 125 to 361 minutes, with an average of 224. We do not say that these patients had no physical disease, but at least there were no other definite, direct clinical signs of it.

Increased familiarity with this test, therefore, has caused us to place increased reliance on it in separating these groups. We are all familiar with the

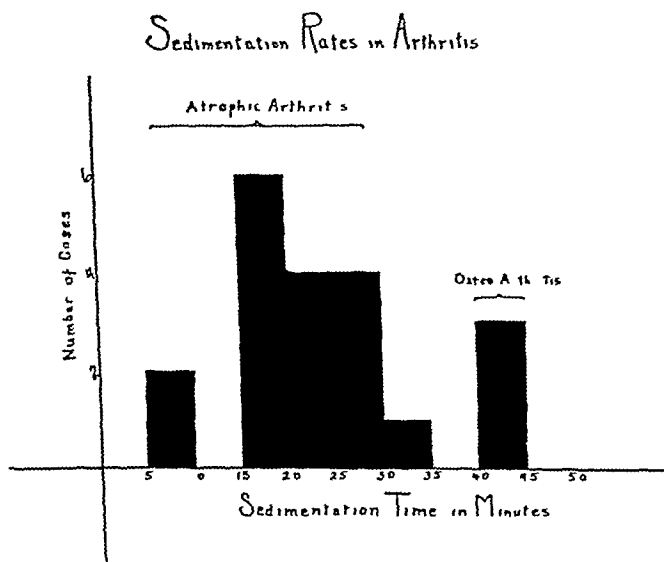


Fig. 2

clinical picture of the patient who complains of pain in the shoulder, hip or arm, and in whom ordinary physical examination, leucocyte and differential count, x-ray, etc., may show no abnormality. Has this patient an early arthritis without physical signs or is he having pain without physical basis? The question frequently is raised in compensation cases and the physician on the witness stand hesitates to commit himself too far in either direction. It is in just this group of cases that the cell sedimentation time has proved of great value.

Following up those cases with a short sedimentation time (an increased rate) without other physical signs we have found in our series that sooner or later they show definite signs of joint involvement which may or may not improve under treatment. And the group with normal sedimentation rates have, in our experience, shown no further clinical signs of organic disease. We feel that the test is of distinct value in this differential diagnosis.

Another group in which the test is of differentiating value is in urologic conditions. The average time we have found to be greatly shortened in pyelitis

(13 minutes), and in cystitis (27.5 minutes), but normal in cases of renal or ureteral calculi without infection (262.2 minutes). Even in the early cases of pyelitis, without prostration or any considerable amount of pyuria the sedimentation time has been distinctly lowered. Seventeen cases of cystitis varied from 13 to 70 minutes, all of them in a low range, averaging 27.5 minutes. Nine cases of hypertrophy of the prostate varied from 13 to 200 minutes, varying obviously with the amount of infection in the bladder accompanying the primary clinical condition.

Chronic or severe acute infections, in general gave a markedly reduced time, e. g., pelvic abscess, ten cases with an average of 24.1 minutes, septicemia seven cases, with an average time of 18.6 minutes, phlebitis three cases with an average time of 24.3 minutes, other localized infections, 19 cases, with an average of 40 minutes.

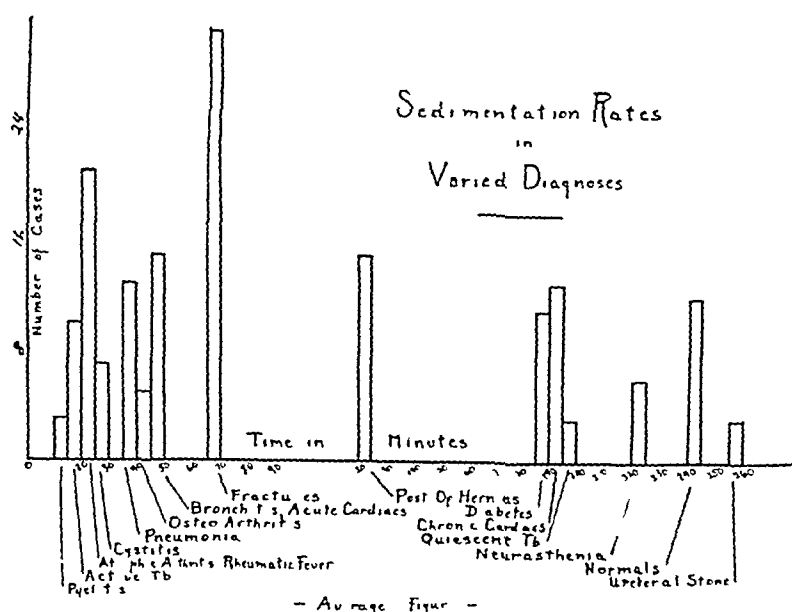


Fig 3

The test has been used in tuberculosis with outstanding results. We had ten proved active cases of tuberculosis, with sedimentation times varying from 6 to 31 minutes with an average of 16.1 minutes. Three cases of quiescent or healed tuberculosis varied from 125 to 316 minutes with an average of 194, that is, about normal.

Thirteen cases of lobar and bronchopneumonia gave cell sedimentation times averaging at 35 minutes. Acute and chronic bronchitis lowered the time to a slightly less degree, as did influenza.

Eleven cases of diabetes varied from 51 to 340 minutes, obviously depending on concurrent factors, for 4 cases with gangrene had reduced times averaging 35 minutes.

Cardiac conditions varied with the type of disease, or conversely the test can be used to separate the acute endocarditis, from that disease where symptoms are

due to old, chronic scarring of the valves. Thirteen cases of chronic valvular heart disease had an average time of 198 minutes, while 7 cases of acute, infective cases averaged at 48.7 minutes. How often we wonder if an old cardiac lesion, with obvious valvular disturbance, has engrafted onto it an acute process, with infection of the endocardium? This test may help us to decide about this point.

The test in cases with tumors varied within wide limits, in the nonmalignant group from 11 to 148 minutes, with an average of 62, and in the 26 malignant cases, from 7 to 221 minutes. This suggests that the test is of less importance in the diagnosis of cancer but is influenced by infection and other extraneous factors.

Fractures generally showed a somewhat reduced time, more sharply so in compound fractures, but present to a less extent in even simple fractures, and gradually returning to normal as the bone healed.

A number of other conditions are listed in the table, where the cases were too few, or the results too variable to draw accurate conclusions. We realize that each of these groups of cases is small and that the conclusions on the "average" figure from such a small group cannot be held too mathematically rigid.

#### CONCLUSIONS

The red cell sedimentation rate is a test that is easily performed and the results easily read.

Normal individuals have a sedimentation time (Linzenmeier method) of over two hours.

Severe acute and chronic inflammatory conditions greatly lower this time.

This is of diagnostic and prognostic value in rheumatic fever, atrophic and osteoarthritis and in differentiating mild degrees of these conditions from functional disturbances, symptomatically similar.

The test is also of value in separating inflammatory from noninflammatory conditions of the urinary tract, and is of considerable confirmatory evidence in many other conditions.

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# STUDIES IN VENOUS PRESSURE\*

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## I HISTORICAL

EARLY descriptions of methods of measuring venous pressure in man were published by Frey,<sup>1</sup> von Basch Gacitner<sup>2</sup> and others. Von Recklinghausen<sup>3</sup> describes the method upon which the instrument by Hooker and Eyster<sup>4</sup> was based for estimations applicable to clinical work. They reported venous pressure in a series of cardiac cases with the use of this instrument. Determinations with the direct method were first made by Moritz and Tabora - Schott<sup>5</sup> and others. These observations compared closely with those found by indirect methods. Many workers have studied venous pressure in relation to its various physical and physiologic aspects. During the past few years, the work of Hooker, Eyster, Middleton and several others has increased the interest in venous pressure and shown its value in clinical medicine. Clark<sup>6</sup> was the first to make a close study of venous pressure in cardiac decompensation and its relationship to prognosis and treatment.

## II PHYSICAL MECHANISM CAUSING VENOUS PRESSURE

With the body at rest the pressure of the blood in the arteries is fairly constant, and the same holds true with regard to the veins. As the heart beats blood is taken from the veins and forced into the arteries, and differences in pressure in the arteries and veins develop, due to resistance offered to the moving stream by the arterioles and capillaries. Physical conditions cause the pressure to be highest at the driving head, that is, the heart, and to fall increasingly towards the end of the system, or the inflow into the heart. The term "peripheral resistance"<sup>7</sup> has been used to explain the marked fall of pressure occurring between the arterial and venous portions of the vascular system. Thus, the two obvious factors, the head of pressure or cardiac output and the variable peripheral resistance, explain the change of pressure in the two sides of the vascular system.

A factor which must be further considered is the peripheral vascular bed, that is, the arterioles and capillaries. It has been shown<sup>8</sup> that abolition of vasoconstrictor control is followed by a marked fall in arterial pressure, but little or no change is noted in venous pressure. Removal of vasoconstrictor tonus by greatly reducing peripheral resistance theoretically should cause a marked rise in venous pressure, but this is compensated for by an increase in the total vascular bed. Splanchnic stimulation causes contraction of the arterioles and capillaries, i. e., a reduction of the vascular bed. This is followed by a slight rise in venous pressure.

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In ordinary conditions the pressure in any superficial vein is due to two factors. The first of these is the influence exerted by the pumping action of the heart, the second the effect of gravity. This latter factor is modified by the influence of skeletal muscular contraction which decreases the caliber of the veins and expresses the blood from them, hence, the venous pressure is reduced. These factors are largely eliminated by having the subject at complete body rest, in the recumbent position, and the vein selected for pressure determination on a level with the entrance to the right heart.

### III METHODS FOR DETERMINING VENOUS PRESSURE

Two methods have been used for determination of venous pressure, the direct and the indirect methods. In the first, a trocar is introduced into the vein and from this a tube extends upward. As the blood comes to a height in the tube the reading is taken in centimeters. This method is probably the more accurate, but for clinical purposes when determinations are made daily or more often, it is less useful than the indirect method which has found greatest favor in clinical work. This method depends upon the principle that if outside pressure is exerted upon a superficial vein, the vein will either be patent or collapsed, depending upon whether the outside pressure is lower or higher than that of the blood within the vein. Many forms of instruments for determining venous pressure by the indirect method have been constructed. Probably the best is the one originally designed by Hooker and Eyster<sup>4</sup> and which has been constructed by J. S. Hipple, Mechanician of the Medical School of the University of Wisconsin, from whom an instrument was obtained for my use.

### IV NORMAL VENOUS PRESSURE AND PHYSICAL CONDITIONS WHICH AFFECT IT

In the determination of venous pressure the patient is at rest in the recumbent posture. In a group of patients without circulatory disturbances it was found that the average venous pressure was 60 mm. of water, the extremes being 35 mm. and 90 mm. Many variations have been given for normal venous pressure with determinations made under the same basic conditions. Frey,<sup>1</sup> the first to use the indirect method, found the extremes in sitting position to be 54 mm. to 179 mm. of water. Hooker<sup>10</sup> reported readings varying from 30 mm. to 150 mm. of water. Runge<sup>11</sup> found pressures between 48 and 66 mm. of water. Readings on numerous normal subjects in the recumbent position by Eyster and Middleton<sup>2</sup> were found to range between 50 mm. and 60 mm. of water, which has been accepted as the normal.

Many factors may influence normal venous pressure. The most important of these has been shown to be posture which causes a rise when the patient changes from the recumbent to the erect posture. It was found that the pressure rises on an average of 20 mm. of water from recumbent to the erect position, ranging from 70 mm. to 90 mm. in the series studied by me.

Muscular activity has been found to be a factor which influences venous pressure. If an estimation is taken (a) with the patient at rest and (b) after he has had a brisk walk, it will be found to increase on an average of 20 mm. to 40 mm. of water. Hooker<sup>12</sup> made observations during vigorous exercise and found an in-

crease of from 70 mm to 140 mm of water Villaret St Gions and Grellity-Bosviel<sup>14</sup> also show an increase after a prolonged walk

The influence of respiratory activity is significant The negative pressure in the thorax during inspiration facilitates the flow of blood into the heart and causes a rise of pressure in the intrathoracic veins The varying negative pressures in the thorax, associated with different phases of respiration, produce correspondingly varying effects on venous pressure<sup>15</sup> This will be the greater, the deeper the respiration During quiet breathing in man it has been found that so far as the pressure in the peripheral veins is concerned, there is little variation between the phases of inspiration and expiration This is due to absorption of the differences encountered in the thorax by the inertia of the blood column between the central and peripheral veins Definite respiratory variations in pressure in a peripheral vein are absent or else very slight during quiet breathing<sup>15</sup> This condition has also been shown to be true by Bedford and Wright<sup>16</sup> and Waud<sup>17</sup> No variations have been found in the cases which have been studied in this series In hyperpnea, respiratory variations became evident and there is a fall of mean venous pressure in the normal subject of 7 mm to 30 mm of water<sup>16</sup> In cardiac decompensation the overventilation tends to lower venous pressure, but even with this factor the pressure is found high

#### V VENOUS PRESSURE IN CARDIAC DECOMPENSATION

This is the one condition in which the majority of workers agree that consistent changes in venous pressure occur Fiey<sup>1</sup> in 1902 stated that a rise in venous pressure may be an early sign of impending cardiac failure Gaertner<sup>18</sup> one year later, noted pressures as high as 230 mm of water in several cases of cardiac decompensation Hooker and Eyster<sup>4</sup> in 1908, reported several cases of decompensation in which venous pressure was at first high, falling to normal as compensation was reestablished Clark<sup>7</sup> in 1915, showed that (1) peripheral venous pressure rises to an abnormal level in this condition, (2) the degree of rise is approximately proportional to the degree of clinical cardiac failure, (3) that such determinations are of value in prognosis and treatment Many workers have shown similar results Villaret, St Gions and Grellity-Bosviel,<sup>14</sup> using the same method, found an abnormally high venous pressure in decompensation and state that a rising pressure may be an early sign of impending heart failure Eyster and Middleton<sup>12</sup> in 1924, reported a series of cases in which the venous pressure maintained a high level during decompensation Eyster<sup>15</sup> in 1929, showed a definite increase in venous pressure during decompensation with a reduction as compensation returned

The mechanism of the increase of venous pressure during cardiac decompensation has been widely discussed It was early recognized that venous pressure may be influenced by the rate at which the heart receives blood from the veins and forces it into the arterial system<sup>8</sup> A reduction in cardiac output such as occurs in vagal inhibition would obviously tend to produce stagnation in the vena cava, and a rise in venous pressure would result Increased cardiac output on the other hand would tend to cause a depletion of the venous blood and a low venous pressure Thus, the cardiac output is a variable factor, which causes changes in

venous pressure. However, a different conception of the relation of venous pressure and cardiac output has developed in recent years. It sets forth the idea that venous pressure is probably the main factor in determining cardiac activity.

These two conceptions started a discussion which has not yet been fully closed. At present, it is the idea of the majority of observers that the two views are interdependent and that cardiac activity, since it is regulated by venous pressure, is a most important factor in tending to stabilize it. When the heart muscle becomes diseased and its power to contract is lessened, a decrease in cardiac output results. This leads to a stagnation of blood in the veins with a rise of venous pressure, edema and engorgement due to blood stasis, supervene. The kidneys show marked congestion, evidenced usually by reduction of urinary output and albuminuria. As congestion decreases, the urinary output increases. This was first shown by Clark,<sup>7</sup> and has been observed in this series also. Eyster<sup>14</sup> in his most recent work has shown that the influence upon the heart muscle due to lack of oxygen supply is of importance. There is less oxygen during decompensation due to the slowing of the pulmonary circulation which results in poor aeration. Thus, the blood in the coronary arteries does not have the proper oxygen supply. Dilatation of the heart results in contractions which are more rapid and less forceful. These produce a further reduction in cardiac activity with its lowered cardiac output and increased venous pressure.

Practically all observers are agreed that a definite increase in venous pressure occurs during decompensation. A series of 35 cases showing decompensation has been studied. It was found that the pressure was high with decompensation, and as compensation was restored, the venous pressure returned to normal. In cases showing steady or persistent increase in venous pressure, death usually occurred. In some of these cases it was found that the venous pressure would remain at a high level for some time and then a further rise would be noted before any definite change could be detected clinically. In patients whose venous pressure readings were of this type, the prognosis was usually grave. At the height of decompensation with its increased venous pressure, it was found that the urinary output was extremely small. As the venous pressure began decreasing, the urinary output became greater. In Table I, a few typical cases are shown.

#### VI VENOUS PRESSURE IN CHRONIC HEART DISEASE

Various observers have shown that unless there is evidence of cardiac failure the venous pressure is normal. A group of 15 cases of organic heart disease who have never suffered decompensation has been studied. The venous pressure in these patients did not exceed normal limits.

The next group of cases of interest are those who have alternate decompensation and compensation. In a study of 15 of these cases during compensation the same results are found as in those who have never had decompensation.

Many of these patients continue to take a maintenance dose of digitalis daily and the amount of their physical activity is extremely small.

#### VII VENOUS PRESSURE IN ARTERIAL HYPERTENSION, NEPHRITIS AND UREMIA

Hypertension has little effect on venous pressure. Fuch<sup>15</sup> found a venous pressure of 56 mm. of water in 6 cases of hypertension. Bedford and Wright<sup>16</sup>

TABLE I

CASE NO	TYPE OF HEART DISEASE	VENOUS PRESSURE	HOSPITAL DAYS	CONDITION	RESULT
1	Syphilitic Aortic insufficiency	140	8	Marked edema, poor	Died
2	Rheumatic Mitral stenosis and insufficiency	110	23	Poor, edematous	Died
		115	to		
		135	30	Worse	
		150	32		
		140		No change	
		140	38		
		140			
		150	39	Worse, edematous	
		160	to		
		170	46	Much worse	
		190			
3	Rheumatic Mitral stenosis and insufficiency, aortic insufficiency	120	19	Poor, edematous	Died
		100	23	Improved	
		90	23	Improved	
			28		
		110	32	Slightly worse	
			to		
		90	38	Improved	
		140	60	Worse, marked edema	
			to		
			70		
		160	80	Very edematous	
4	Arteriosclerotic and hypertensive	120	10	Poor, very edematous	Compensated
		125	to		
		110	15		
		120	25	Unchanged	
		110	to		
		90	40	Improved	
		90	60	No edema but not completely compensated	
5	Arteriosclerotic	160	2	Very poor	Compensated
		170	5	Worse	
		130	10	Much improved	
		110	15	Improved	
		90	20	Improved	
		70	25	Improved	
6	Arteriosclerotic and hypertensive Auricular fibrillation	130	2	Fairly good	Compensated
		120	5	Moderate edema	
		90	10	Improved	
		80	14	Compensated	
7	Arteriosclerotic Auricular fibrillation	110	31	Edema, fair	Compensated
		110	34	Edema, fair	
		100	38	Improved	
		90	42	Compensated	
		80	46	Compensated	
8	Infectious with cardiac hypertrophy and dilatation	140	6	Moderate edema, fair	Compensated
		110	10	Improved	
		80	12	Compensated	
		60	14	Good	
9	Arteriosclerotic and thyroid Auricular fibrillation	180	1	Extremis, edema 4+	Compensated
		160	3	Slightly improved, poor	
		140	5	Improved, edema 2+	
		130	8	Improved, edema 2+	
		120	12	Improved, edema +	
		120	14	Improved, edema slight	
		120	16	Improved, edema slight	
		100	18	Good, no edema	
		90	20		
		80	21	Fine, no edema and compensated	

TABLE I (CONTINUED)

CASE NO	TYPE OF HEART DISEASE	VENOUS PRESSURE	HOSPITAL DAYS	CONDITION	RESULT
10	Rheumatic, mitral stenosis, auricular fibrillation	120	3	Moderate edema, poor	Compensated
		100	5	Slight decrease in edema	
		90	10	Improved	
		90	12	No edema	
11	Arteriosclerotic and hypertensive	160	1	Marked edema	Compensated
		140	4	Slight improvement	
		120	8	Improved	
		100	10	Improved	
		80	14	Compensated	
12	Syphilitic, aortic insufficiency	130	4	Poor, very marked edema	Died
		120	7	Improved	
		100	8	Improved	
		90	10	Good, no edema	
	Patient developed auricular tachycardia	100	12	Slightly worse	
		120	13	Much worse	
		140	14	Edema	

TABLE II

VENOUS PRESSURE		
1	Rheumatic heart disease Mitral stenosis and aortic stenosis and insufficiency	90
2	Rheumatic heart disease Aortic insufficiency with mitral insufficiency	80
3	Rheumatic heart disease Mitral stenosis	80
4	Rheumatic heart disease Mitral stenosis	70
5	Syphilitic heart disease Aortic insufficiency	90
6	Rheumatic heart disease Mitral stenosis	80

have also found normal pressure. In our group of 10 cases of arterial hypertension without evidence of heart failure, it has been found that there is only a slight increase above the average normal. Thus, we believe we can say that in this type of vascular lesion venous pressure shows little or no change. Types of these cases are shown in Table IV.

It has been possible to observe several patients with marked hypertension, severe nephritis, and uremia. These patients had edema of the lower extremities but the clinical picture was not that of cardiac failure. Only 4 such cases have as yet been followed through, so no conclusions are made. No explanation is offered other than to suggest the possibility that with decrease of kidney function and appearance of edema the results may be comparable to those seen in cardiac failure. The venous pressure was high when first taken and increased until the patient died.

TABLE III

TYPE OF HEART DISEASE	VENOUS PRESSURE
Syphilitic	
1	80
2	80
Arteriosclerotic and hypertensive	
1	80
2	60
3	80
4	80
5	90
6	70
7	80
8	80
9	80
Rheumatic	
1	80
2	70
3	90
4	70

TABLE IV

TYPE OF DISEASE	VENOUS PRESSURE	BLOOD PRESSURE
Arteriosclerosis and hypertension	80	190/100
Chronic nephritis		
Arteriosclerosis with chronic nephritis	80	200/100
Arteriosclerosis and hypertension	90	210/100
Arteriosclerosis	90	200/135
Chronic nephritis		

TABLE V

TYPE OF DISEASE	VENOUS PRESSURE	BLOOD PRESSURE	RESULT
General vascular sclerosis	150	250/150	Died
Chronic nephritis			
General vascular sclerosis	140 to 180	180/120	Died
Chronic nephritis			
General vascular sclerosis	140 to 170	210/160	Died
Chronic nephritis			
General vascular sclerosis	120 to 180	190/120	Died
Chronic nephritis			

## VIII VENOUS PRESSURE IN LOBAR PNEUMONIA

One of the diseases most frequently complicated with circulatory failure is pneumonia. It is found that lobar pneumonia has no effect on venous pressure until circulatory failure occurs. However, it is shown that by frequent determina-

tions the appearance of failure can be foretold by a rise in venous pressure before it is evident clinically Eyster<sup>15</sup> says that as long as the heart is sufficient and a normal circulation is maintained, the venous pressure remains normal There is a rise in pressure as in cardiac decompensation when the heart fails Increase of venous pressure is the first indication of failing heart Eyster and Middleton<sup>12</sup> reported several cases of lobar pneumonia of which 6 showed a venous pressure within normal limits The other cases showed an increased venous pressure and evidences of cardiac failure Fuchs,<sup>10</sup> in 9 cases of lobar pneumonia, showed 8 to have normal venous pressure Two of these patients died within thirty-six hours without further determinations being made The other case had a high venous pressure and died Kastlin and MacLachlan,<sup>10</sup> in a series of 90 cases of lobar and bronchial pneumonia, showed that a majority of these patients with normal venous pressure recovered, the mortality being 14 per cent The majority of their patients with slightly increased venous pressure recovered, the mortality being 11 per cent The majority (61 per cent) of cases with high venous pressure died The cases showed very little evidence of circulatory failure except increased venous pressure This series agrees with the previous observers, that an increase of venous pressure in pneumonia is evidence of circulatory failure and such determinations are very valuable in prognosis, as the usual signs of cardiac failure are not seen at the time when the pressure begins to rise

#### IX. THERAPY IN VENOUS PRESSURE

It is extremely difficult to separate the influence upon venous pressure in cardiac decompensation of drug therapy, bed rest and limitation of diet and foods No drug is known which in the normal animal will act as a specific to reduce venous pressure<sup>21</sup> Digitalis and caffeine used in normal animals showed a rise in arterial pressure but no change in venous pressure Morphine and strychnine caused no change<sup>21</sup> Nitrites caused a fall In man, inhalation of amyl nitrite caused a slight increase<sup>16</sup> The use of digitalis has long been known to be of value in cases of cardiac decompensation Its action is to decrease the rate and increase the force of the beat If we consider the conception of heart rate as a factor in venous pressure, we must believe that digitalis is of value in decompensation by reducing rate, increasing force of driving head, reducing venous pressure and causing the signs of decompensation to disappear In a group of cases of cardiac decompensation studied, a combination of drugs has been used It is, of course, impossible to say that any one thing is responsible for the results It is believed that digitalis is of value Every case studied received this drug in some form It was found that in a few cases of abnormal rhythm, auricular fibrillation, tachycardia, etc., the signs of decompensation returned unless the maintenance dose of digitalis was continued for some time

Diuretics of some form were used in most of these patients The results from theocin and theocalm were practically the same Salrgan was found to be quite valuable in the reduction of edema, with a consequent decrease in venous pressure It was found to be of more value and somewhat less apt to cause bad effects than novasurol The best treatment for decompensation was a combination of bed rest morphine, limited diet and fluids digitalis, and later salrgan The majority of the patients recovered compensation



Venesection in the treatment of cardiac decompensation has long been used. Clark<sup>7</sup> used this method and showed that after venesection the venous pressure was reduced but that it rose again very rapidly. Eyster<sup>15</sup> has shown that high venous pressure was reduced definitely after venesection for a long period of time. If the load of increased pressure on the heart can be reduced for a short time, the factors causing decompensation may be abated and compensation reestablished rapidly. In some of the cases reported by Eyster<sup>15</sup> there was definite improvement and subsequent recovery. Others died within forty-eight hours, showing no improvement. The greater number of the cases showed improvement and for this reason it is believed that venesection is of definite value in the treatment of cardiac decompensation.

#### A. SUMMARY AND CONCLUSIONS

In this study of venous pressure several factors have been discussed. From these, certain conclusions may be drawn. The physiologic mechanism affecting venous pressure has been summarized. The factors which affect venous pressure in normal individuals, the changes of venous pressure in cardiac disease and associated conditions have been listed. It has been shown that the normal venous pressure taken under basic conditions as described is about 60 mm of water. In organic heart disease unless there is evidence of failure, the venous pressure does not exceed the normal limits. As cardiac failure appears, it may be indicated by a rise in venous pressure before other clinical evidence is present. A continued high venous pressure or a rising pressure during treatment is a bad prognostic sign. High arterial pressure alone causes little or no change in venous pressure. With chronic nephritis and uremia the venous pressure is increased. In pneumonia, unless cardiac failure occurs, venous pressure does not show an increase. An increase in venous pressure in this disease is the first evidence of cardiac failure. Reduction of venous pressure in cardiac decompensation and the restoration of compensation cannot be attributed to any one therapeutic measure but to the combination of several.

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## THE SPECIFIC GRAVITY OF THE BLOOD DURING SPINAL ANESTHESIA\*

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THE following is a report of the specific gravity of the blood during spinal anesthesia. The determinations were made by the delicately accurate falling drop technic of Barbour and Hamilton<sup>1</sup> "*Principle* A drop of blood (or other body fluid) of definite size is released below the surface of a nonmiscible mixture. Its rate of fall depends on its density, which can be easily calculated as soon as the rate of fall of a similar drop of standard solution of known density (released under identical conditions) is available for comparison."

A total of twenty anesthetics were studied. Twelve only serve as a basis of this report, there was one death shortly after the administration of the spinal anesthetic, five others were supplemented by gas or ether and in two no control readings were obtained. Thus these twelve cases, carefully worked up, represent smooth anesthetics from beginning to end, in which blood specific gravity determinations were made before and during the operations. However, it may be stated here that eighteen of the cases followed the same general pattern described below.

These observations were gathered over a period beginning October, 1926, and ending in March, 1928. We had contemplated working up a larger series but various things prevented this. However, we feel that these few cases were carefully controlled and are worthy of presentation. The perspective obtained by the elapsed time has aided us in the proper evaluation of our findings.

*General Analysis*—Table I represents the manner in which our data were collected. There were three general patterns discernible and these are depicted in graph form in Charts 1, 2 and 3. Table II shows at a glance the composite findings in the nine cases not graphed.

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\*From the Gynecologic Service, Parnert Hospital.  
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Attention is invited to the falling time of the drop of blood in that it was found to be a more sensitive indicator of changes in blood density than when the falling time was interpolated into terms of specific gravity. To one not accustomed to determining the specific gravity of the blood, a change of one point in the third decimal place may seem insignificant, but to the person clocking off the falling time on a stop watch split to tenths of a second, a change of one to twenty seconds is quite impressive. Normally, the specific gravity of the blood from moment to moment is so constant that a change of even one point in the third decimal place must be considered as heralding a definite, though slight and transitory change in the status of the blood. To make the tables more comprehensible the nearest half second of falling time was used. Likewise, only the second and third decimal values (to the nearest half of the fourth decimal value) of the specific gravity of the blood are used. Thus a reading of 1.0532 is represented by figure 53, a reading of 1.0535 is represented by the figure 54.

TABLE I  
BLOOD DENSITY AND BLOOD TENSION DURING SPINAL ANESTHESIA\*

TIME A M		TENSION	FALLING TIME		BLOOD DENSITY
BP	BD		BLOOD	STANDARD	
9 57		104/72	17.7	18.6	10560
10 03	10 05	88/64	20.4		10533
	10 09		21.9		10529
10 14	10 16	84/62	21.7	18.5	10531
	10 25		23.0		10511
10 28	10 29	84/60	20.8		10527
10 35	10 36	84/64	20.9	18.4	10526
10 45	10 46	86/64	20.6		10529
	10 57		20.5		10530
10 58	10 59	154/92	20.4		10531
11 06	11 07	182/98	19.9		10535
11 08	Operation ended				

\*Case 1, October 1, 1926. Female, aged fifty. Vaginal repair. Control (prior to operation).  
B P 172/78 B D 10552

a Pattern I, Chart 1.—In a previous publication one of us<sup>2</sup> made preliminary mention of the specific gravity of the blood during spinal anesthesia. Table I is a reproduction of the protocol published at that time. The statement was made then “that with the fall in blood tension that follows the administration of the spinal anesthetic there is a concomitant fall in blood density, it being noted that as the patient begins to react from the anesthesia the rise in blood tension is followed by an increase in blood density.” We have had no reason to change this comment since this is what we are pleased to term the “usual or general pattern” obtained. One fact may be noted here, to be discussed later, is that in Cases 1, 10, and 19, there is a preliminary rise in blood density during the first five minutes of the anesthesia, even though the blood pressure has begun to fall.

b Pattern II, Chart 2.—In this pattern there was no immediate transitory increase in blood density. There were, rather, immediate wide and sweeping fluctuations downward and upward, the general trend being consistently parallel with the change in blood tension.

TABLE II

COMPOSITE FINDINGS IN NINE CASES OF SPINAL ANESTHESIA

Case 1										Case 2										Case 3										Case 4										Case 5										Case 6										Case 7										Case 8										Case 9										Case 10										Case 11										Case 12										Case 13										Case 14										Case 15										Case 16										Case 17										Case 18										Case 19										Case 20										Case 21										Case 22										Case 23										Case 24										Case 25										Case 26										Case 27										Case 28										Case 29										Case 30										Case 31										Case 32										Case 33										Case 34										Case 35										Case 36										Case 37										Case 38										Case 39										Case 40										Case 41										Case 42										Case 43										Case 44										Case 45										Case 46										Case 47										Case 48										Case 49										Case 50										Case 51										Case 52										Case 53										Case 54										Case 55										Case 56										Case 57										Case 58										Case 59										Case 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c Pattern III, Chart 3—In two of the twelve cases the blood pressure sounds were inaudible. These were recorded as zero pressures. Babcock<sup>3</sup> in 1922 stated that "a fall in the systolic pressure in the radials to zero is occasionally seen." Of course, the radial systolic pressure cannot actually fall to zero, since such zero pressures are incompatible with life. In the one death mentioned, the blood pressure

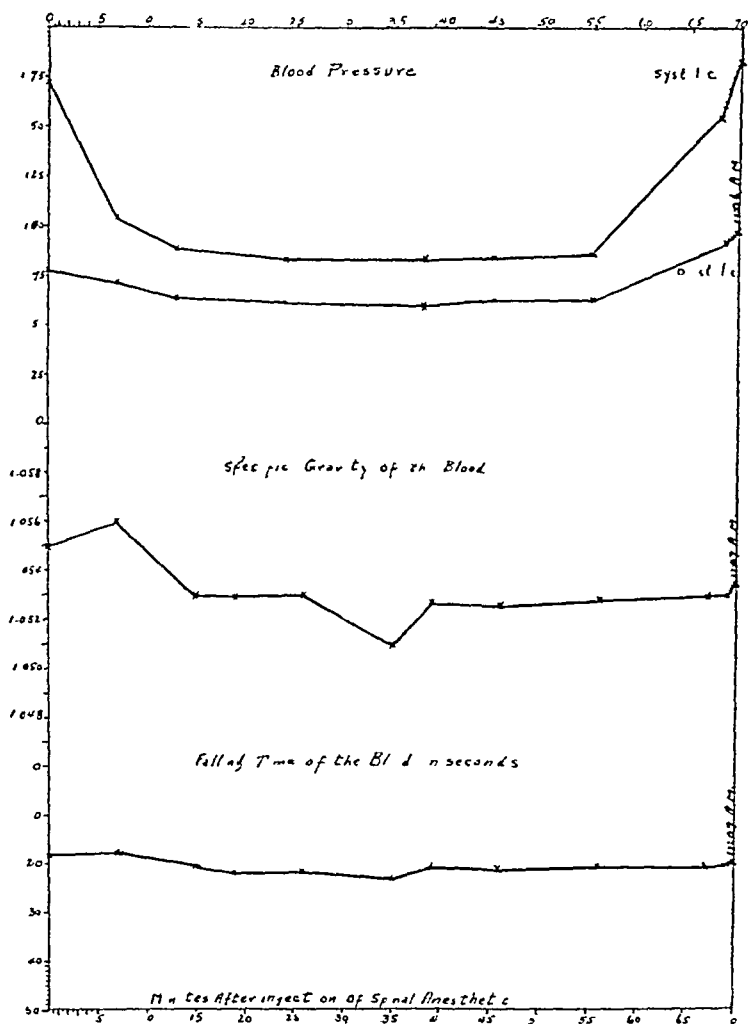


Chart 1—Case 1, Oct. 1, 1926, female aged fifty, vaginal repair. Spinal anesthesia, administration at 9:50 A.M.

fell to zero and the exceedingly high specific gravity of the blood of 1.067 was obtained. The blood densities in these two cases followed the usual downward trend, but on the recovery of the blood tension the blood density was inclined to fluctuate in steeper gradients upward.

In general, then, it was found that the greatest fall in systolic pressure took place in five to twenty-two minutes after the administration of the anesthetic, the greatest fall in diastolic pressure in five to twenty-six minutes, the greatest fall in the specific gravity of the blood in fourteen to thirty-three minutes.

As an additional comment on blood pressure and blood density in general, one gains an increasing respect for the constancy of the diastolic pressure and the latter's possible immediate effect upon the blood density

Note also was made of the fact that in seven cases vomiting or eructations took place at the depths of blood tension and blood density



Chart 2—Case 9 March 18 1927, female, aged fifty-five perineorrhaphy Spinal anesthesia, administration at 9 24 A M

#### DISCUSSION

Perhaps the only value these observations may have is that they throw a small additional light on the question of shock. No human experimental method so closely simulates the condition of shock as does spinal anesthesia. This is especially true in that the latter and true shock have one great thing in common, namely the extreme hypotension. The surprisingly good chemical condition of patients under spinal anesthesia is comparable to the work of Sherrington (cited by Macleod\*) on spinal shock in monkeys. Sherrington found that in a monkey whose spinal cord

had been cut far forward, the posterior part of the animal was in shock, there was hypotension, while the monkey amused himself by catching flies with his fore limbs

Any discussion of blood density presupposes and accepts the idea that any change in the blood is at once manifested by a change in its specific gravity. Such a change may be brought about by an alteration in the number of erythrocytes

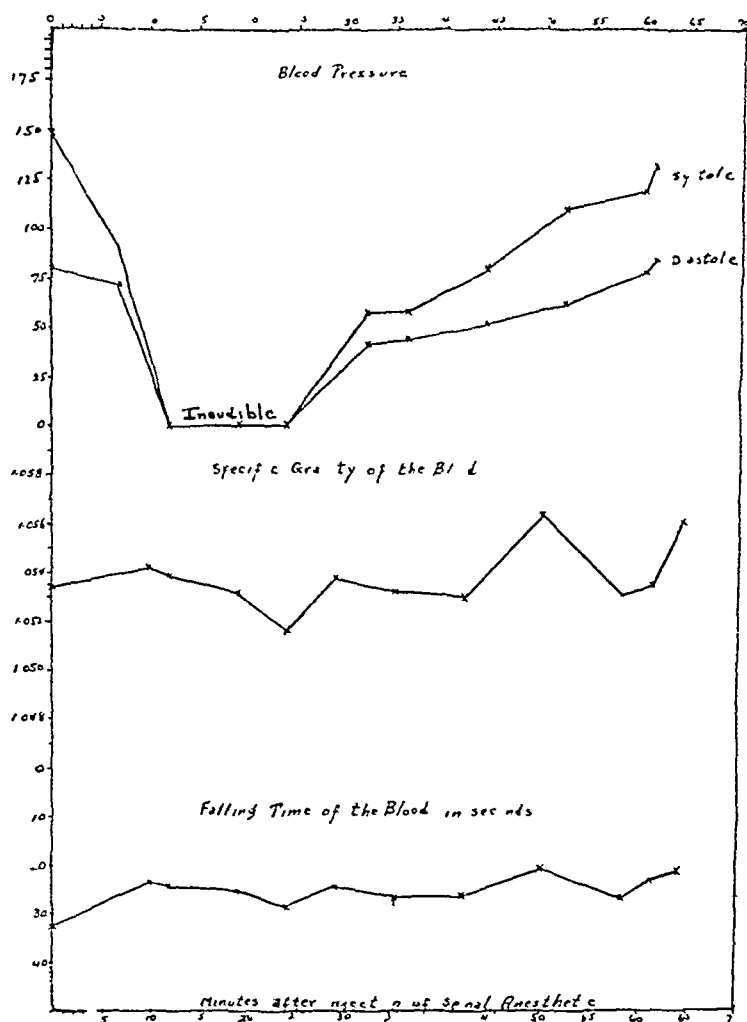


Chart 3—Case 10 female aged fifty perineorrhaphy, October 1927 Spinal anesthesia administration at 10 12 A M

leucocytes, platelets, by alteration of the hemoglobin content, fibrinogen content, ash content, by shifting water balances (viscosity), by alteration of lipid values. Since this work shows that the blood density is consistently lowered during spinal anesthesia and since it is thought that a greater blood pressure is required to propel more viscous than less viscous blood, we are of the opinion that the lowered blood density is a protective mechanism. The lowered blood density is a response to the lowered blood pressure which is thus better able to propel the blood through the body. It is recognized that the reverse may be the case.

The transitory rise in blood density at the beginning of some of the spinal anesthetics may be due to a concentration of the blood, a jamming back of blood in the capillaries. A concentration of the blood in this manner is said to precede the condition of true shock.

As one studies the tables and charts it is discernible that the end stages of the anesthesia are ushered in by a wave-like change in blood density. This is a definite phenomenon which the less sensitive mechanism of blood pressure does not always record. One gets the feeling that the patient is rising out of the depths of anesthesia in undulating fashion, and not in the easy gradual way which a picture of the blood pressure alone would indicate.

#### SUMMARY

- 1 The results of blood density determinations in twelve cases of spinal anesthesia are recorded and an attempt made to analyze them.
- 2 It appears that three general patterns are obtainable with the dominant finding that of parallelism of blood density with blood tension. A lowered blood tension is accompanied by a lowered blood density.
- 3 The suggestion is advanced that this lowered blood density is a protective mechanism.

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### ETIOLOGIC STUDIES IN HODGKIN'S DISEASE\*

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THE nature and the etiology of Hodgkin's disease are still in doubt. Bacteriologic studies have given divergent results which have been interpreted variously. Steinberg<sup>1</sup> attributed it to a modified or attenuated form of the tubercle bacillus. Reed<sup>2</sup> described the histologic picture in the nodes and called attention to the frequent association of tuberculosis and Hodgkin's disease sometimes even to be found in the same node. Fraenkel and Much<sup>3</sup> described a granular, nonacid fast bacillus seen in sections from the nodes. Negri and Mieremet,<sup>4</sup> and independently Bunting,<sup>5</sup> cultivated a diphtheroid bacillus from Hodgkin's tissue. Bunting and Yates<sup>6</sup> inoculated monkeys with these organisms. The blood picture and lymphoid involvement in these resembled Hodgkin's disease. Many workers have cultivated diphtheroid organisms from Hodgkin's lymph nodes (Billings and Rose-  
now,<sup>7</sup> Kusumaki,<sup>8</sup> Litterer,<sup>9</sup> Lanford,<sup>10</sup> Olitsky,<sup>11</sup> Rhea and Falconer,<sup>12</sup> Ives,<sup>13</sup> Mel-

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lon,<sup>14</sup> Bloomfield,<sup>15</sup> Torrey,<sup>16</sup> and Steel<sup>17</sup>) Mellon, in a bacteriologic study of seven strains of organisms isolated from Hodgkin's nodes demonstrated that they differed from each other in staining, vigor of growth, resistance to antifoaming, involutionary forms, pigment formation, and other biologic characteristics. Similar organisms have been cultivated from the lymph nodes in conditions other than Hodgkin's disease (Lanford, Yates, Bunting and Kristjanson,<sup>18</sup> Harris and Wade,<sup>19</sup> Steel). Torrey showed that different strains of diphtheroids could be cultivated from the same lymph node of Hodgkin's disease. Bloomfield announced a bacterial flora for normal and diseased nodes. Cunningham<sup>20</sup> and Fox<sup>21</sup> believed that no one organism with definite morphologic and cultural characteristics had been isolated from Hodgkin's nodes. Agglutination and complement fixation tests using organisms isolated from Hodgkin's nodes and the serum from patients with the disease have been practically negative (Kristjanson,<sup>22</sup> Ebeison,<sup>23</sup> Moore,<sup>24</sup> Torrey, Bloomfield, Ohtsky and Lanford). Administration of a vaccine made from the organisms isolated has no more curative effects than injection of foreign protein (Billings and Rosenow, Mellon Moore, Wallhauser and Whitehead,<sup>25</sup> Litterer and McVey and Wilson<sup>26</sup>). While lesions similar to Hodgkin's disease have been reported in lower animals, (MacFadyen,<sup>27</sup> Hodgson,<sup>28</sup> Simons,<sup>29</sup> Reid,<sup>30</sup> Yates,<sup>31</sup> Simonds,<sup>32</sup> Jobling<sup>33</sup>) Rolleston<sup>34</sup> is of the opinion that more definite evidence from histologic examination is necessary before the occurrence of Hodgkin's disease in animals can be accepted. Nonconfirmatory reports have followed the attempt to produce the disease in horses, monkeys, rabbits, guinea pigs, and chimpanzees (Reed, Lanford, Rhea and Falconer, Mellon, Bloomfield Torrey, Moore, Cunningham and McAlpin,<sup>35</sup> Stewart and Dobson<sup>36</sup> and DeLeon and Reyes<sup>37</sup>). L'Esperance<sup>38</sup> reported the production of the disease in chickens by the intravenous injection of macerated lymph node, and believed it to be due to the avian tubercle bacillus. Twort<sup>39</sup> was unable consistently to demonstrate a specific animal or vegetable parasite in the affected tissue from Hodgkin's disease, either by direct microscopic examination, cultivation or animal inoculation (rabbits and guinea pigs). Nor did his assortment of *in vivo* or *in vitro* experiments give any positive results. His researches extended over a period of six years and included very elaborate studies on 61 cases. Waithin<sup>40</sup> considered Hodgkin's disease to be a malignancy. More recently Medlar<sup>41</sup> has suggested that it is a neoplasm originating in bone marrow.

It was with the hope of securing additional evidence that the present experimental studies were undertaken. Lymph nodes from five cases presenting the clinical features of Hodgkin's disease were used. The diagnosis was confirmed by microscopic examination. They contained typical lymphadenoma giant cells, eosinophiles, plasma cells, and endothelial overgrowth, fibrosis and mitotic figures. Two of the nodes were obtained as biopsy specimens, and three at postmortem examination. To avoid extraneous contaminants the surfaces of the nodes were first seared with a hot spatula. Then with sterile instruments a small piece of tissue was removed from the center through the seared surface.

Bits of this, about 0.1 gm. in weight, were crushed and planted in brain broth, dextrose broth, hormone broth, melted plain agar, brain broth in deep tubes, blood agar, ascitic fluid, and broth and agar enriched with ascitic fluid. Cultures were incubated for thirty days before discarding. The cultures from one case remained sterile. In one case there was a diphtheroid bacillus and a motile bacillus and a

variety of other organisms which were regarded as contaminants. From three cases a variety of diphtheroid bacilli were isolated. An identical organism occurring in each of the three cases was made the subject of further study. They were apparently identical in all particulars. This organism was markedly pleomorphic, showed curved granular rods 2-4  $\mu$  in length and coccoid forms 0.6  $\mu$  in length, V-shaped, dumbbell and bottle forms were seen, but no banded forms. It stained well with Loeffler's methylene blue. By Gram's method the granules stained positively but the matrix varied in its retention of the dye. It was nonacid fast and nonmotile. In the cultures it required ten to fourteen days to make its appearance. A low oxygen tension was required for its growth which was obtained by planting the tissue at the bottom of a deep culture tube filled with media, or by the use of sodium hydroxide and pyrogallol acid. After several months' cultivation this preference for low oxygen tension gradually disappeared and the organism became completely aerobic. The colonies in agar were small, round, grayish white, mainly discrete, measuring 0.5 to 2 mm. in diameter. Daughter colonies forming at right angles to the stab appeared rapidly after twenty to thirty days' incubation. These were larger, less sharply circumscribed and less opaque than the earlier ones. In the shallow broth cultures grown under partial anaerobic conditions there developed a clouding of the lower third of the media and a heavy sediment at the bottom. No acid or gas was formed on mannite, galactose, saccharose, glucose, levulose, maltose or lactose. It did not acidify or coagulate litmus milk, produced no indol, no pigment nor did it liquefy gelatin. Bloomfield,<sup>15</sup> Torry,<sup>16</sup> and others have described a similar organism isolated both from normal and from diseased lymph nodes. The latter gave it the name *Bacillus lymphophilus* because of the frequency with which he encountered it in lymph nodes.

Following the techniques outlined by Hiss and Zinsser<sup>44</sup> agglutination, complement fixation, bacteriolytic and precipitation tests were made using this organism and serum from patients with Hodgkin's disease and from normal controls. No demonstrable agglutinins, precipitins, complement fixing bodies or bacteriotropic substances were demonstrated. For the complement fixation tests the blood serum was obtained from three patients with Hodgkin's disease in whom the Wassermann reactions were negative. The control sera used were from three normal individuals with negative Wassermann's and from three syphilitics with positive Wassermann's. No fixation of complement with antigen prepared from the organism in question occurred with any of these sera. For bacteriotropic substances, the blood serum of patients with Hodgkin's disease was added to cultures of the organism. Also Beikfeld filtrates of Hodgkin's lymph nodes were added to the cultures with negative results.

Animals were inoculated in some cases with the organism just described, in other cases with bits of lymph nodes aseptically obtained. The organism was introduced in a variety of ways. Following the method described by Moon and Konzelmann<sup>42</sup> cotton plugs saturated with a heavy suspension of living organisms were implanted under aseptic precautions through a trocar into the peritoneal cavity and into the subcutaneous tissue. Bits of sterilized bone were allowed to incubate in the cultures for several days and were then implanted as were the cotton plugs. Salt solution suspensions of the organism broth and soft agar cultures of it were injected intravenously, intraperitoneally into and beneath the skin. The

animals were carefully watched for clinical manifestations. Routine temperature readings and blood counts were made. Weights were checked frequently. The postmortem examinations of the animals were made with aseptic precautions. Postmortem bacteriologic studies of the lymph nodes, spleen, liver, kidney, blood and foci of infection were made routinely. Specimens from the lymph nodes and visceral organs were fixed in 10 per cent formalin and Zenker's fluid, cut and stained with hematoxylin and eosin and also with carbol fuchsin in certain cases.

Monkey (*macacus rhesus*) No. 1 received a total of three intraperitoneal inoculations of the organism, two subcutaneous implantations in the cervical region, of bone impregnated with the organism, and implantations in the neck of fresh lymph node substance. Following subcutaneous inoculations the regional lymph nodes became slightly swollen but rapidly subsided. Bi-weekly blood counts were within normal limits.<sup>43</sup> The blood sedimentation time averaged between 3 and 4.5 mm. in one hour. Temperature records and clinical data were normal. The animal was permitted to live for eleven months and was healthy when destroyed. Except for a chronic proliferative foreign body reaction about the cotton plugs and about the implanted bone, no lesion was demonstrated by gross or microscopic examination. Cultures from spleen, lymph nodes, kidney, and cotton plugs were sterile. A coccus was cultivated from the liver.

Monkey (*macacus rhesus*) No. 2 was inoculated intraperitoneally with the organism. Two and one-half months later he was killed with ether and a careful examination was made. There were no gross or microscopic lesions. The organism inoculated was isolated from a mesenteric lymph node and from the spleen.

Four half grown chickens of the Plymouth Rock variety were inoculated as follows. Two received subcutaneous implantations of bone impregnated with the organism cultivated from Hodgkin's nodes. These lost their appetites, lost weight and became anemic. One died one and a half months later, the other two and a half months later. At the postmortem examination no gross or microscopic lesion was demonstrated. The organism injected was not recovered. No acid fast organisms were demonstrated in any of the tissues. Two chickens were inoculated subcutaneously with pieces of fresh lymph node from Hodgkin's disease. Seven and a half months later they were healthy and were killed and examined. No gross or microscopic lesion was demonstrated. Sections from all the organs were examined but no acid fast organisms were found.

Two healthy half grown dogs were inoculated intraperitoneally with a heavy suspension of the organisms on cotton plugs introduced through a trocar. The dogs were observed for six months before they were destroyed. Clinically they showed no evidence of disease. Temperature and blood counts showed no significant variations. The blood sedimentation time was within normal limits. At the postmortem examination no gross nor microscopic changes were demonstrable in the lymphoid structures nor elsewhere. Cultures were made from the cotton plugs and from the various tissues. The inoculated organism was not recovered.

As a routine diagnostic procedure guinea pigs were inoculated with pieces of lymph nodes from each case. In addition five guinea pigs were injected intravenously and intraperitoneally with freshly isolated cultures. Preceding inoculation the animals had received intravenous and intraperitoneal injections of India ink for the purpose of blocking the reticulo-endothelial system and rendering the

animals perhaps more susceptible to infection. They lived from two to five months. At the postmortem examination no lesions of Hodgkin's disease nor of tuberculosis were found.

Sixteen rabbits were inoculated as follows. Crushed lymph nodes were implanted intraperitoneally on a cotton plug in two. In eleven the organism was introduced in the various ways described earlier in this article. In one the animal received an intravenous injection of India ink prior to the implantation of the organism. Two were inoculated with the substance of fresh Hodgkin's lymph nodes and cultures of the organism. The animals receiving large amounts of freshly isolated organism died in less than a month with progressive loss of weight, anorexia and paralysis of hind quarters. The others were destroyed six months following inoculation. There were no signs of Hodgkin's disease during life. At postmortem examination there were found in the tissues no lesions resembling Hodgkin's disease.

#### COMMENT

In four out of five cases of Hodgkin's disease studied, a variety of pleomorphic bacilli were cultivated from the lymph nodes. Experiments designed to demonstrate the relationship between one of these organisms present in each of three cases, and Hodgkin's disease were carried out. No agglutinins, precipitins or complement fixing bodies were found in the blood serum of patients suffering with Hodgkin's disease. Inoculation of this organism and the Hodgkin's lymph nodes into monkeys, guinea pigs, chickens, dogs and rabbits failed to reproduce the disease. Cultures made at the postmortem examination of the artificially produced foci of infection, the lymphoid structures and other visceral organs demonstrate that in some of the animals the organism persisted in the foci and spread to other organs, that in others it persisted only in the foci, while in still others no evidence of infection nor of persistence of the organism at the site of inoculation resulted.

It is established that with appropriate technique bacilli of the genus *Corynebacterium* can be isolated from Hodgkin's tissue. There is no sound experimental evidence that these organisms bear any etiologic relationship to the disease. The cultivation of similar organisms from pathologic lymph nodes, the seat of a variety of lesions, is further evidence against this relationship. The only significant association possible is that as in typhoid and leprosy they might be the cause of the disease but do not reproduce it in animals. Study by all other known methods does not confirm this.

Inoculation of pieces of fresh Hodgkin's nodes and of the organisms cultivated from them into chickens failed to produce either Hodgkin's disease or avian tuberculosis. Postmortem bacteriologic cultures and histologic examination of their tissues failed to reveal acid fast organisms. As it is difficult to get laboratory fowl which are free of avian tuberculosis it is probable that L'Esperance<sup>38</sup> mistook this common finding for significant experimental results. Branch<sup>39</sup> suggests that she was dealing not with Hodgkin's disease but with avian tubercle bacillus infection in the human being.

#### CONCLUSIONS

A variety of organisms of the genus *Corynebacterium* can be cultivated from a high percentage of cases of Hodgkin's disease. An identical organism cultivated from three cases has been studied experimentally.

No precipitins, agglutinins, complement fixing bodies, or bacteriotropic substances for this organism were found in the blood serum of patients suffering with Hodgkin's disease

Monkeys, chickens, dogs, guinea pigs, and rabbits were inoculated in a variety of ways with this organism and with pieces of fresh Hodgkin's lymph nodes. In none of them did the lesions of Hodgkin's disease develop.

Neither avian tuberculosis nor lesions resembling Hodgkin's disease were found in fowl inoculated with cultures or with fresh tissue from Hodgkin's disease.

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## THE EFFECTS OBSERVED FOLLOWING THE INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION OF FLUID AN EXPERIMENTAL STUDY ON DOGS\*

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THE common practice of forcing the administration of fluid without specifying either the amount or the time interval is mexact, and in certain instances, may be harmful. Attention has been directed to this fact by Lewis,<sup>1</sup> de Takats,<sup>2</sup> Hunt, McGann, Rowntree Voegtlm<sup>3</sup> and others.

Any method which would permit the clinician to determine accurately either a deficit or an excess of water in the tissues of a patient, from time to time, would be a desirable addition to modern methods of treatment.

The experimental work which forms the basis of this report was started for the purpose of finding such a method. We also wished to observe if large quantities of fluid produced harmful results and if so under what conditions. The aims have not been realized as yet but certain observations have been made which are significant.

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The literature concerning the blood changes in conditions affecting the body water balance is voluminous. Klein and Nonnenbruck<sup>4</sup> found that in subjects with normal metabolism intraduodenal infusion of water regularly caused a reduction in the number of erythrocytes in arterial blood. Boycott and Douglas<sup>5</sup> gave hypertonic glucose solution to normal and nephritic rabbits and found that the latter took a longer time to adjust themselves. Several deaths were attributed to cardiac failure which were said to occur more frequently in the nephritic than in the normal rabbits. Smith and Mendle<sup>6</sup> injected an amount of fluid, which they considered equal to the blood volume of a rabbit, and then by repeated blood counts and hemoglobin determinations, found that the bulk of the fluid had left the blood stream in the first five minutes. This fluid could not be accounted for by passage into the muscles or by edema. The fluid in the serous cavities and that in the stomach and intestines therefore as well as the volume of urine are concerned with the disposal of fluid leaving the circulation. There is considerable disagreement as to the fate of the fluid injected. Dastie and Love<sup>7</sup> found that 75 per cent of the fluid injected intravenously passed into the serous cavities and tissue spaces. MacCallum<sup>8</sup> injected large volumes of fluid intravenously and from a cannula tied into the lumen of the intestine, obtained up to 15 per cent of the injected fluid.

Keith, Rowntree, and Geraghty<sup>9</sup> in a series of articles, the first published in 1915, introduced the dye method of determining blood volume. This has since been elaborated upon and values have been determined for numerous conditions, experimental and clinical. Lampson and Rosenthal<sup>10</sup> declare that true blood volume, plasma volume or red cell volume cannot be measured by any of our methods. They give reasons for the belief and conclude that it is impossible by the present methods to differentiate between blood changes due to fluid loss and those the result of vasomotor or circulatory changes. One of these authors in collaboration with others later stated that on account of the inability to determine the amount of red blood cells and fluid lost or gained, it is impossible to interpret the results obtained in terms of fluid exchange between blood and tissues even though 25 c c of normal salt solution is given per kilogram of body weight. They found that volumes determined by means of vital red and hemoglobin did not always agree.

#### METHODS

Well nourished, apparently normal, adult dogs were used for the experiment. Isotonic sodium chloride solution was administered subcutaneously in amounts of 2000 c c or intravenously in an amount of 1500 c c. Observations on the following factors were made both before and after the administration of fluid: (1) Physical response, (2) blood volume changes, (3) blood cell counts, (4) hemoglobin concentration, (5) total blood proteins, (6) tissue thirst, (7) electrocardiograms, (8) urinary changes. These observations were repeated on animals in which renal damage had been produced, nephrectomies performed, or diphtheria toxin administered, for the purpose of producing cardiac damage.

Blood volumes were determined by the vital red method, as described by Hooper, Smith, Belt, and Whipple.<sup>11</sup>

Before any fluid was given the average blood volume of each experimental animal was established by two or more estimations at intervals of from five to ten days. Blood volume estimations were repeated immediately after intravenous administration of fluid and two hours after subcutaneous injection.

Total serum proteins were determined by means of the micro refractometric method, using the Zeiss dipping refractometer

Skin wheals were produced by the introduction, intradermally, of 0.2 cc of normal salt solution and were used on a small series of dogs

Electrocardiograms were run on a series of animals, before and after the administration of fluid

Urinary volumes, specific gravities and the presence or absence of albumin were determined following the introduction of fluid

To check the dye method of blood volume determination, two dogs were bled and perfused

To produce myocardial damage, diphtheria toxin was given, following the method described by Stewart<sup>12</sup> We found it necessary to reduce the dosage by half in order to keep the animals alive for a sufficient length of time to complete the observations Renal damage was produced by clamping the vessels bilaterally as done by Ivy<sup>13</sup> and associates Total nephrectomies were done on two dogs

#### RESULTS ON NORMAL ANIMALS

**Blood Volume**—The results obtained on the effect of the intravenous injection of 1500 cc of isotonic sodium chloride solution, during a period of fifteen to twenty minutes, on the circulating blood volume of dogs as determined by the vital red method are shown in Table I No increase in circulating blood volume that could be measured by the dye method occurred

**Blood Counts and Hemoglobin Determinations**—It was found that five minutes after the intravenous administration of 1500 cc of 0.9 per cent sodium chloride solution, the red cell count and hemoglobin content were decreased significantly, the average decrease being 23 per cent The maximum and minimum decrease varied in the different dogs from 10 to 36 per cent, but in the same dog from time to time the decrease did not vary more than 10 per cent

TABLE I

SHOWING THE EFFECT OF THE ADMINISTRATION OF 1500 CC OF 0.9 PER CENT SODIUM CHLORIDE SOLUTION INTRAVENOUSLY ON BLOOD VOLUME DETERMINED BY THE DYE METHOD

DOG NO	CONTROL BLOOD VOLUME DETERMINATIONS*		POST INJECTION BLOOD VOLUME DETERMINATIONS	
	NO TRIALS	AV BLOOD	NO TRIALS	AV BLOOD (CC)
4 157 Kilo	5	1418	1	1397
5 118 Kilo	5	1118	4	1128
6 105 Kilo	2	1097	1	923
7 19 Kilo	5	1367	2	1365
3 205 Kilo	8	2117	1	2127

\*Variations from time to time were less than  $\pm 100$  cc



These results indicated a blood dilution following the injection of fluid, and were at variance with the results cited above. This apparent dilution occurred so uniformly that we questioned the accuracy of the dye method for determining total blood volume under the conditions of our experiment. Consequently, we checked the vital red method with the method of bleeding and perfusing in an animal to which no fluid had been given and in another after the administration of 1500 c c of isotonic salt solution. We found that the results obtained by the dye method checked to within 2 per cent in the normal animal but failed to check in the hydrated animal. The volume obtained by bleeding and perfusing was greater than when vital red was used for the determination. The value determined by bleeding and perfusing corresponded closely to the increase indicated by the blood count, hemoglobin and hematocrit determinations.

The injection of 2000 c c of isotonic sodium chloride solution subcutaneously had no effect on the red cell count two hours after the injection and before complete absorption had taken place.

*Hematocrit Determinations*—Hematocrit readings five minutes after the intravenous administration of 1500 c c of fluid revealed an average dilution of nearly 20 per cent. Although there were variations of from 10 to 20 per cent in different animals the values in the individual animal remained fairly constant. The dilution persisted for at least one hour but had disappeared at the end of twenty-four hours.

*Total Blood Proteins*—A study of the total blood proteins before and after the intravenous administration of isotonic salt solution shows that they were decreased from 20 to 52 per cent in the various dogs. In the same dog from time to time the maximal variation of the decrease was 12 per cent. In Table II are shown the results in which the total blood proteins were followed in three dogs up to one hour after the injection of fluid. From this table it will be seen that the total proteins do not return to the prefluid level within one hour.

TABLE II

SHOWING THE TOTAL BLOOD PROTEINS BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF 1500 C C OF 0.9 PER CENT SODIUM CHLORIDE SOLUTION

	DATE	PRE FLUID VALUE	IMMEDIATELY FOLLOWING FLUID	15 MIN. LATER	1 HR. LATER	PER CENT DECREASE
Dog 5	12 11	7.3	4.3	5.6	5.6	39
Wt	1 12	6.9	4.2	4.8	4.9	39
11.8 K	1 22	6.5	3.8	4.9	4.9	41
	3 2	6.5	3.1	4.1	4.5	52
Dog 4	12 17	6.7	4.9	5.8	5.8	36
Wt	1 14	7.1	5.6		6.3	47
15.7 K						
Dog 7	12 3	7.1	5.6		6.3	21
Wt	12 6	6.9	5.1	5.7	6.4	26
19 K	1 13	7.5	6.0	6.1	6.5	20

In Table III is shown typical data on three dogs in which all determinations were made as nearly simultaneously as possible. This was not always technically feasible. Three determinations on Dog 5 are shown to illustrate the variations en-

countered in the same dog. The only striking discrepancy is in the decrease in total proteins in the case of Dog 5, tests "A" and "C," in which the blood counts and hematocrit figures checked, but the decrease in total proteins is approximately twice what it should be on the basis of blood dilution alone. The only explanation of this discrepancy that occurs to us is that some protein has actually left the circulation, which does not usually happen.

*Tissue Thrust*—Skin wheals were produced by introduction of 0.2 cc of 0.9 per cent sodium chloride solution intradermally. Several hundred readings were made at various times under varying conditions. From the data gathered no definite conclusions could be drawn due to the extreme variability of the length of time required for the wheal to flatten to the level of the surrounding skin.

*Urine*—A large number of urinary outputs were studied. After the production of kidney damage and after the administration of diphtheria toxin, there was a one plus albumin in the urine but this was reduced to a trace following the injection of fluid.

TABLE III

COMPOSITE CHART SHOWING THE CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1500 CC OF ISOTONIC SODIUM CHLORIDE SOLUTION

		BLOOD VOLUMES	BLOOD COUNTS	TOTAL* PROTEINS	HEMATOCRIT**
Dog 4 Wt 15.7 K	Prefluid	1418 cc	7,010,000	6.7	59
	Postfluid	1397 cc	6,240,000	5.8	65
	Per cent decrease		12	13	10
Test A Dog 5 Wt 11.8 K	Prefluid	1118 cc	4,320,000	7.3	58.8
	Postfluid	1233 cc	3,350,000	4.3	77
	Per cent decrease		23	47	25
Test B Dog 5	Prefluid	1118 cc	7,920,000	6.9	
	Postfluid	1016 cc	5,120,000	4.2	
	Per cent decrease		35	39	
Test C Dog 5	Prefluid	1118 cc	6,300,000	6.5	60
	Postfluid	1016 cc	5,010,000	3.8	77
	Per cent decrease		21	41	23
Dog 7	Prefluid	1391 cc	7,040,000	7.5	58
	Postfluid	1548 cc	5,720,000	6.0	71
	Per cent decrease		18	20	19

\*Percentage of total serum protein

\*\*Percentage of serum in whole blood

Volumes listed as prefluid are the average of a series of normal volumes as shown in Table II

There was a tendency toward lowered specific gravity in the dogs that had had the renal vessels clamped but the return to normal within twenty-four hours was just as regular as in normal dogs. This was true in animals given salt solution on three successive days.

*Results on Animals Given Diphtheria Toxin*—Four dogs were given diphtheria toxin in an attempt to produce myocarditis. Observations were then made on these animals before and after the administration of fluid.

*Blood Volumes*—It is evident from the results (Table IV) that diphtheria toxin tends to cause a decrease in blood volume which is partly, but not entirely accounted for by the loss of weight. In two of the three dogs given 1500 cc of salt solution there was an increase in blood volume following the first injection. Later however no increase resulted.

*Blood Cell Counts, Total Protein and Hematocrit*—As will be seen from Table IV there is no marked difference in the response to fluids given intravenously, to a dog suffering from the effects of diphtheria toxin and a normal animal.

Table V shows a comparison of the total proteins before and after the administration of isotonic salt solution in a dog which had received diphtheria toxin, one that had the renal vessels clamped for thirty minutes, fourteen days previously and

TABLE IV

SHOWING THE EFFECT OF DIPHTHERIA TOXIN ON NORMAL BLOOD VOLUME AND ON THE BODY MECHANISM FOR CONTROLLING THE BLOOD VOLUME AFTER THE INTRAVENOUS ADMINISTRATION OF 1500 CC OF ISOTONIC SALT SOLUTION

DOG NO	NORMAL BLOOD VOLUMES		1 2 WEEKS AFTER DIPHTHERIA TOXIN			FOLLOWING INJECTION OF NaCl SOLUTION		
	NO TRIALS	BLOOD (C C)	NO TRIALS	DAYS AFTER	BLOOD (C C)	NO TRIALS	DAYS AFTER	BLOOD (C C)
1 5.7 Kilo	2	648	2	30 37	499 405	DIED DURING FIRST INJECTION 41ST DAY		
3 20.5 Kilo	8	2117	2	5 15	1600 1764	2	9 12	2432 1380
6 10.5 Kilo	2	1097	1	1	946	1	4	923
7 19 Kilo	5	1367	1	14	1298	2	18 22	1737 1092

TABLE V

SHOWING THE PERCENTAGE OF TOTAL PROTEINS BEFORE AND AFTER THE INJECTION OF ISOTONIC SALT SOLUTION

	PREFLUID VALUE	IMMEDIATELY FOLLOWING FLUID	15 MINUTES LATER	1 HOUR LATER
a*	8.7	4.0	5.3	5.4
b	6.5	3.8	4.0	4.5
c	6.4	3.1	3.8	4.6

\*a—A dog 18 days after the administration of diphtheria toxin

b—A dog suffering renal damage

c—A bilaterally nephrectomized animal

a bilaterally nephrectomized animal Comparing these results with those of Table II it will be seen that they are about the same as those shown in the normal dog

*Electrocardiograms*—Electrocardiograms were made on normal animals and then repeated following the administration intravenously, and subcutaneously, of isotonic sodium chloride solution

When fluid was given subcutaneously one striking result was obtained, namely, lowered voltage in all leads It was found that this change in voltage corresponded roughly to the amount of fluid administered and that as the solution was absorbed, the height of the waves returned to their normal level

There was no evidence of an arrhythmia, or of any disturbance of conduction or of changes in the axis deviation

Electrocardiograms were made before, during and after the intravenous injection of 2000 c c of 0.9 per cent sodium chloride solution In only one case did they show a result similar to that recounted above, with lowered voltage in all leads, and in this one case it was not marked The rhythm, rate, and contour of the curves were unchanged

The administration of fluid to the dogs given diphtheria toxin, resulted in no change which would distinguish the graphs from those of normal animals

*Physical Findings*—Five liters of 0.9 per cent sodium chloride solution were injected subcutaneously into a fourteen pound dog The skin over the trunk was

TABLE VI

EFFECT OF 0.9 PER CENT SODIUM CHLORIDE, GIVEN INTRAVENOUSLY, TO A DOG SUFFERING FROM THE EFFECT OF DIPHTHERIA TOXIN

	BLOOD COUNTS	TOTAL PROTEIN	HEMATOCRIT
Prefluid	9,130,000	7.4	58
Postfluid	6,460,000	5.5	71
Per cent decrease	29	27	20

tense and would pit on pressure, but there was no edema of the extremities When allowed to walk around the room the animal was ataxic, probably from the amount of fluid he was forced to carry He salivated and began almost immediately to urinate Auscultation of the heart at this time did not reveal any arrhythmia or other unusual findings Twelve hours later he had entirely recovered, was playful and apparently had suffered no bad effects from the experience

Sodium chloride solution was administered intravenously to normal dogs in amounts up to 2000 c c, fifteen to twenty minutes being taken for the injection

Following this procedure there was no edema to be noted There was no evidence of fluid in the chest, the lungs were clear, and there were no râles or adventitious sounds The heart rate was slightly accelerated and the rhythm was normal

*Pathologic Changes*—Gross Postmortem examination made on the dogs which had received diphtheria toxin showed nothing of unusual importance The heart appeared somewhat decreased in size but the muscle did not show any gross changes nor did the heart valves The lungs were not changed except in one case in which pneumonia was present In the dogs that had had the renal vessels clamped

there were numerous adhesions to surrounding tissues but the kidneys were grossly unchanged. No enlargement of the spleen was noted in any dog coming to autopsy following the administration of fluid.

Significant findings in the dogs suffering from the effect of toxin into which fluid had been injected were surprisingly lacking. There was some evidence of increased free fluid in the body cavities but except for one dog that contracted pneumonia there were no changes in the lungs. This was also the case in the dog whose kidney vessels had been clamped off bilaterally and in a dog whose kidneys had been removed two days previously. The intestines were usually filled with fluid and the abdominal muscles in the dogs that had had diphtheria toxin were soft and flabby both in those which had and had not received isotonic salt solution intravenously.

#### DISCUSSION

The original aim of this study was that some light might be thrown upon the safety of administering large quantities of fluid and the hope that some relatively simple index might be found that could be used as a warning before the margin of safety was passed.

Large amounts of fluid were given to normal and pathologic animals and no demonstrably harmful effects were seen. There was no increase in blood volume, as measured by the dye method, no persistent change in the urinary output and no changes in the electrocardiograms, which might be interpreted as indicating danger. Because there were no evident effects, no index was found that would indicate the amount of fluid that could be safely administered. However, blood dilution did occur on the basis of red cell counts and hematocrit and blood protein determinations, which had no detectable effect on the normal animal.

The amount of fluid given was proportionately far in excess of that amount usually given patients, and the time of administration was much more rapid than that usually employed with human beings whether given subcutaneously or intravenously. The fluid administered intravenously was not given over a period of several days, which might have caused a breakdown in elimination. However, there was no evidence of any such breakdown in dogs given three liters of isotonic salt solution subcutaneously, on three successive days.

The only unusual result obtained in the study of the electrocardiographic tracings, taken before and after the administration of fluid, was that the voltage was decreased by the introduction of 0.9 per cent sodium chloride solution subcutaneously. This fact should be of clinical importance in cases where edema of varying grades is presented and where lowered amplitude of the waves is taken to indicate myocardial weakness.

In the dogs given diphtheria toxin the results did not entirely correspond to those reported by Stewart<sup>12</sup>. This author interpreted a progressive lowering of the voltage of the electrocardiogram as indicating myocardial damage. The dogs of our series showed lowering of voltage as their condition became worse but in two instances this voltage returned to normal, in one of these the day before the dog died.

The urinary changes were not very significant. They showed that the urinary output alone could not account for the rapid elimination of the fluid from the cir-

culating blood following the intravenous administration of fluids and also that the lowering of serum proteins in the blood was not accounted for by the elimination of protein in the urine. No evidence of permanent damage to the concentrating power of the kidney was shown.

The index of tissue thirst as determined by the McClure-Aldrich skin test was totally unreliable in a large series of dogs.

Although the fluid injected was usually at body temperature, the few times when it was below this level, produced no ill effect except coarse tremors when given intravenously. The speed of injection seemed to cause no difficulty and although no blood pressure tracings were taken, any shock which might have been produced by as much as 100 c.c. per minute for fifteen to twenty minutes, caused no apparent severe physical reaction, nor were there any deaths which might be attributed to this cause.

It was noted that accompanying the increase in urinary output there would be an increase in salivation and at times the dog would vomit fluid. At postmortem there was a large amount of fluid in the gastrointestinal tract. It would seem that this avenue of escape would have an important bearing on postoperative dilatation of the stomach where large quantities of fluid are given.

The studies of blood volume as determined by the dye method occupied a major interest in the experiment. The value for individual blood volumes being relatively constant, it was thought that if any changes could be shown to occur following the administration of fluid, that this change could be used as an index. Since taking a presumed normal from an average figure, found by other investigators, did not give a reliable value for an individual animal and because different animals of the same weight vary as to blood volume, it was thought that the best way to establish a normal was to determine repeated volumes on the same dog. The average of these was then taken as a normal, providing the variance was close to 100 c.c. Usually the variation was not this great. The number of determinations varied from three to ten or more.

An attempt was made to determine repeated blood volumes on the same day according to the method of Smith,<sup>14</sup> but we were unable to get reliable results.

After a normal had been established 0.9 per cent sodium chloride solution was injected and blood volume determined immediately. As can be seen by the preceding tables, there was no appreciable change in the blood volume as determined by the dye method, either on normal dogs or on those animals suffering from cardiac or kidney damage. It will be seen from Table III that a simultaneous check was made on blood dilution as indicated by the blood counts, hemoglobin, and hematocrit determinations, and the total protein content. These values indicate, without question, that there was a definite dilution of the blood following the administration of fluid. This was at total variance with the blood volume values given by the dye method.

It is almost impossible to explain the dilution of the blood, by storage of the red cells in the spleen, because the spleens seen at autopsy were of normal size. It seems unlikely that there would be such a vast storage in the liver, although this is a possibility.

It was also shown that the blood volumes determined by the dye method and perfusion in the dog which had received no fluid checked very closely. There was

not a similar check, however, in the blood volumes determined by dye and perfusion, when an animal had been given fluid intravenously. Here the perfusion method showed a dilution not shown by the dye method. This fact is difficult to explain. One possibility is that the dye method measured only the circulating volume, and that with the addition of large quantities of fluid this circulating volume is kept at a constant level by the shunting of blood into capillary beds which at the time are temporarily closed off from the active circulation, so that they do not form a part of the circulating blood volume. No proof of this hypothesis is offered.

Another possibility is that more of the dye passes out into the tissues or is fixed by the reticuloendothelial cells under normal conditions than when fluid is administered.

#### CONCLUSIONS

1. No harmful results were demonstrated when large quantities of 0.9 per cent sodium chloride solution were given either subcutaneously or intravenously to normal or pathologic animals.

2. Electrocardiograms did not show any characteristic changes when large quantities of fluids were given intravenously either in normal animals or in those presumably suffering from myocardial damage.

3. Isotonic salt solution when injected subcutaneously, decreases the amplitude of the "R" wave in the electrocardiogram.

4. In normal animals exsanguination and perfusion give blood volumes which correspond very closely to those given by the dye method.

5. There is an increased concentration of the blood in animals suffering from the effect of diphtheria toxin.

6. The blood volumes as determined by the dye method show no increase after the intravenous injection of large amounts of fluid. This does not agree with the amount indicated by the dilution of the blood as shown by repeated determinations of the erythrocytes, hemoglobin, serum proteins and plasma increase.

7. No index was found which might be used as a warning against over hydration of tissue.

8. It is believed that the hematocrit is the most simple test for blood dilution following the administration of fluids postoperatively. Its use would be limited, obviously to those cases not manifesting hemorrhage or evidence of rapid hemolysis.

We wish to thank Drs. William H. Holmes, James G. Carr, and A. C. Ivy for many helpful suggestions.

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# LABORATORY METHODS

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## THE PREPARATION OF COLLOIDAL GOLD SOLUTION\*

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THE preparation of colloidal gold solution is, no doubt, one of the most disappointing, time consuming and uncertain procedures encountered in routine laboratory work. Difficulties are numerous as probably has been observed by every laboratory man.

Persistent endeavor with a repeated check up on all known formulas for the preparation of colloidal gold solutions seems to have solved the problem for us.

We have found that the chief trouble was obtaining the proper deep red and stable color, most solutions turning out purple. This was overcome by adjusting the  $P_H$  value of our distilled water. Also the control tube would generally not be decolorized in one hour using 17 c.c. of a 1 per cent salt solution. In adjusting colloidal gold solutions one must take into account that the sensitiveness of these solutions depends on two factors, namely the size of the colloidal particles and the  $P_H$  value of the gold solution, and that this sensitiveness must be maintained.

The method we have adopted for the preparation of colloidal gold solutions is simple, time saving and most satisfactory as tests on known positive as well as negative spinal fluids have shown this solution to be not only reliable but also fairly stable, more so than any other solution purchased previously from Laboratory Supply Houses.

### METHOD OF PREPARATION

To 500 c.c. freshly distilled water (Precision Still) with a  $P_H$  of 6.8-7.0 add 5 c.c. of an aq. 1 per cent gold chloride solution and 25 c.c. of freshly prepared 0.5 per cent potassium carbonate sol (Meick's Blue Label).

Bring to boil, remove from flame and then add while shaking vigorously a few drops of Meick's Neutral Formaldehyde, diluted to about 10 per cent with distilled water, drop by drop. Three to five small drops are usually sufficient. A deep red color develops almost immediately. Cool and let stand tightly corked until next day when it is ready for titration.

### TITRATION OF COLLOIDAL GOLD SOLUTION

Set up 11 Wassermann tubes and place in each tube 17 c.c. of salt solution of increasing strength, ranging from 10 per cent to 20 per cent. For instance the first tube receives 17 c.c. of a 10 per cent salt solution, the second 17 c.c. of a 11 per cent salt solution, the third tube 17 c.c. of a 12 per cent salt solution, and so on until the eleventh tube is reached which receives 17 c.c. of a 20 per cent salt

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solution The various salt solutions can be prepared from a 20 per cent solution as follows

Tube No	1	10 cc	20% salt sol	and 10 cc	H <sub>2</sub> O
Tube No	2	11 cc	20% salt sol	and 09 cc	H <sub>2</sub> O
Tube No	3	12 cc	20% salt sol	and 08 cc	H <sub>2</sub> O
Tube No	4	13 cc	20% salt sol	and 07 cc	H <sub>2</sub> O
Tube No	5	14 cc	20% salt sol	and 06 cc	H <sub>2</sub> O
Tube No	6	15 cc	20% salt sol	and 05 cc	H <sub>2</sub> O
Tube No	7	16 cc	20% salt sol	and 04 cc	H <sub>2</sub> O
Tube No	8	17 cc	20% salt sol	and 03 cc	H <sub>2</sub> O
Tube No	9	18 cc	20% salt sol	and 02 cc	H <sub>2</sub> O
Tube No	10	19 cc	20% salt sol	and 01 cc	H <sub>2</sub> O
Tube No	11	20 cc	20% salt sol	and 00 cc	H <sub>2</sub> O

Each tube now contains 20 cc of a salt solution of the various percentages from 10 to 20 per cent Mix well and discard 0.3 cc from each tube, thus leaving 17 cc Now add to each tube 5 cc of the freshly prepared colloidal gold solution The tube, containing the lowest percentage of salt and showing complete decolorization after one hour, indicates the strength of the salt solution to be used For instance if decolorization of 5 cc colloidal gold solution occurs in the tubes containing the higher percentage of salt solution down to and through 12 per cent in one hour, but no decolorization below this percentage, then the 12 per cent salt solution is the solution to be used in the control (twelfth) tube and 0.4 of this strength or 0.48 per cent salt solution in the other 11 tubes

TABLE I  
SHOWING RESULTS OF 107 SPINAL FLUID EXAMINATIONS

NO OF CASES	DIAGNOSIS	CELL COUNT	GOLD CURVE	WASS	BLOOD	
					WASS	KAHN
65	Normal	35	Normal	Neg	Neg	Neg
14	Syphilis	Increased	Syphilitic	4 plus	4 plus	4 plus
1	Syphilis	Increased	Syphilitic	3 plus	4 plus	4 plus
1	Syphilis	Increased	Syphilitic	Anticcompl	4 plus	4 plus
1	Syphilis	3	Syphilitic	Neg	4 plus	3 plus
3	Paresis	Increased	Paretic	4 plus	4 plus	4 plus
1	Encephalitis	8	Syphilitic	Neg	Neg	Neg
1	Encephalitis	5	Syphilitic	Neg	Neg	Neg
1	Encephalitis	12	Syphilitic	Neg	Neg	Neg
1	Encephalitis	75	Syphilitic	Neg	Neg	Neg
1	Encephalo		Center			
	Meningitis	1288	Curve	Neg		
1	Brain Tumor	58 3050	Normal	Neg	Neg	Neg
4	Meningitis	Increased	Meningitic	Bact Exam	meningococci	
4	Meningitis	Increased	Meningitic	Bact Exam	pneumococci	
3	Meningitis	Increased	Meningitic	Bact Exam	streptococci	
5	Tubercle					
	Meningitis	Increased	Meningitic	Guinea Pig	Inoculation	
					Positive t b	

It will be seen from Table I of cases that 15 definitely syphilitic cases with strongly positive Wassermanns on both spinal fluid and blood, also with positive blood Kahn reactions, gave typically syphilitic type curves with the colloidal gold reaction Two other syphilitic cases one with a negative and the other with an anticomplementary reaction in the spinal fluid Wassermann tests, but with strongly positive blood Wassermanns, gave typical luetic curves with the colloidal gold

Three cases, diagnosed as paresis, with strongly positive spinal fluid and blood Wassermanns and positive blood Kahn reactions, all gave definitely positive parietic curves with the colloidal gold

Four cases of encephalitis with negative spinal fluid and blood Wassermanns and Kahn reactions gave syphilitic curves with the colloidal gold

It has not been unusual in our experience to obtain typically syphilitic type curves in cases clinically diagnosed as encephalitis with the spinal fluid and blood Wassermanns negative

One case, diagnosed as encephalomeningitis, and with a high cell count gave a center curve a little to the right of a typical syphilitic type curve and extending into the meningitic curve region

One case with a brain tumor with negative spinal fluid and blood Wassermanns gave no alteration from the normal with the exception of a high and varying cell count

Sixteen cases, clinically diagnosed as meningitis and bacteriologically proved as such, all gave typical meningitic type curves

The other cases reported checked with the negative clinical diagnosis of syphilis and the negative spinal fluid and blood Wassermanns as well as Kahn reactions and gave normal colloidal gold curves

Such findings are not possible unless the point of decolorization and sensitiveness of the colloidal gold solution is accurately balanced. This balance is essential and accurate titration seems to be indicated, no matter what method is used in the preparation of the colloidal gold solution

The method herewith submitted for the preparation of this solution is not original with us but was adopted as the best from our experience

Titration of the colloidal gold solution, to determine the correct percentage of salt solution to be used, will help solve the difficulty that many laboratories have had and are having in its preparation

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## THE PHARMACOLOGIC STANDARDIZATION OF PARATHORMONE\*

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THE assay of parathormone (parathyroid extract Collip), or other parathyroid extracts, due to the peculiar criteria required to measure their potency, is comparatively difficult. Collip and other investigators<sup>1</sup> have shown that the active principle of this material quantitatively affects the mechanism controlling the calcium and phosphorous concentration within the blood stream. The first of these physiologic processes to be employed, and the one which is now believed to be the most reliable for the purpose of measuring the degree of activity of parathyroid extract, is concerned with blood serum calcium. Collip, Clark, and Scott,<sup>2</sup> using a modified Kramer-Tisdall method,<sup>3</sup> determined the serum calcium values in parathyroidectomized dogs. By means of subcutaneous injections of parathyroid

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extract, they were able to maintain a calcium level which prevented the death of these animals. Their next step was an attempt to influence the serum calcium of normal animals. They were able to do this quantitatively on healthy, untreated dogs.

*Test Subjects*—In selecting experimental animals for the routine testing of parathormone, the requirements of the chemical test to be used must be kept in mind. The collected blood must not be contaminated by extraneous calcium. Care must be taken, therefore, that it does not touch the hair or skin of the subject or the hands or clothing of the operator. This requirement eliminates the possibility of collecting blood from an incised vein in the cat, rabbit, guinea pig, rat, or mouse. Cardiac puncture, likewise, is impractical since the procedure, always dangerous, causes frequent deaths and consequent changes in the identity of the test group. Collip<sup>4</sup> found that the response of normal rabbits to parathyroid extract, even in massive doses, was not uniform.

On the other hand, it was found that healthy dogs maintain a fairly constant normal calcium level, are relatively long lived, and can be obtained of such a size that the withdrawal of 20 c c of blood would not appreciably affect the total blood volume over a twenty-four-hour test period.

*Selection of Dogs*—Parathormone is evaluated by the degree to which it is capable of producing a rise in the blood serum calcium of normal dogs. The calcium response of each individual, therefore, must be fairly uniform. In selecting the members of a group for this purpose, it is necessary to exclude all those which might be expected to show gross inconsistencies at any time. This classification includes all females, since a variable response is usually obtained during the estral cycle, old males, because senility reduces tolerance, very young males, because the calcium picture during rapid growth usually shows great variations, and males with respiratory, intestinal, or cutaneous diseases. Or, to state the case positively, a good test dog for parathormone should have the following qualifications:

Sex	Male (mature)
Weight	15-25 kg
Health	Free from gross evidence of disease

It is our experience that, from 100 dogs collected indiscriminately, approximately 20 are suitable for parathormone testing.

*The Individual*—Parathormone unitage is based directly upon the milligram rise of calcium per 100 c c of blood serum. Therefore, the individual when injected with a given number of units of the substance should show an increase in blood calcium, commensurate with the size of the dose.

One unit of parathormone is defined as one one hundredth of the amount required to cause a rise of 5 mg of calcium per 100 c c of blood in a 20 kg dog. It is obvious that one unit would produce a change too small to be of any value and at the other extreme 100 units may produce results which are injurious to the health of the test animal. It has been found that a dose of 66 units causes no injury and gives the most consistent results. If 20 unit material is used, this dose is 3.3 c c per 20 kg dog and should be responsible for a blood calcium rise equal to 0.66 of 5 mg or 3.3 mg per 100 c c of blood serum.

This is the standard or theoretical normal rise for the individual. All dogs

do not respond alike but they should be consistent from week to week and show a rise equal to the normal plus or minus 2. An increase of less than 1 mg of calcium per 100 c c of blood serum should not be considered.

Occasionally one member of a group will show no rise after the customary dose of parathormone. This individual must be replaced in the group by one whose response has proved to be fairly constant. The dog failing to show the specified calcium rise must not be used for testing until his unusual behavior has been corrected. It is often the less expensive course to discard such an animal immediately.

*The Group*—A parathormone test group consists of not less than 5 dogs, but may be made up of as many more as the amount of material to be tested, the cost of the animals, and the time available will permit.

The extract in doses sufficient to cause a rise of 3.3 mg of calcium per 100 c c of blood serum is responsible in some cases for a long continued hypercalcemia. It is advisable, on this account, to use a group but once each week, and it is preferable to use it not more often than once every two weeks.

When a group has been standardized by the injection of a stock preparation of known value, a certain amount of regularity must be maintained in the matter of calcium stimulation to get the most consistent results. The animals should receive a dose of parathormone, whether it be test material or stock, at their regular injection time. The amount of this dose must be sufficient to give 3.3 mg of calcium rise per 100 c c of blood serum, or a dose of 3.3 c c of 20-unit parathormone per 20 kg of dog weight.

*Diet and Environment*—A diet constant in composition and in amount per dog, seems to be essential. Any marked change in either of these factors affects the calcium which is available to parathormone influence, even though the normal remains the same. A prepared diet, containing dried meat, cod liver oil, molasses, dried buttermilk, wheat germ meal, wheat bran, corn cereal, oat cereal, barley malt, and salt, is used in our kennels with complete satisfaction. An occasional raw bone and fresh vegetables may be added to keep the dog's appetite healthy.

A minimum of exercise, and habituation to new living conditions are to be kept in mind, also, when using new dogs.

*Bleeding and Injection*—Blood is usually collected early in the morning of the day on which the dogs receive an injection of the extract. Then feeding is omitted after this bleeding until the final collection has been made on the following morning.

*Apparatus* Glass syringes, 10 c c capacity  
1.25 in., 19 gauge hypodermic needles  
Glass centrifuge tubes, 15 c c capacity, ungraduated  
Rubber stoppers to fit centrifuge tubes

The dog to be bled is placed on a table and held on his side by two assistants. No other means for holding a dog quietly and securely has been found satisfactory. The hair is clipped for a distance of 4 to 6 inches from the ankle upward on the lateral surface of either hind leg. Tourniquet-like pressure, applied at the knee by one of the assistants, will cause the saphenous vein to stand out prominently. This vein will be found to run in a diagonal course from the posterolateral aspect of the knee down to and across or around the ankle. With pressure on the vein

maintained, blood is withdrawn by inserting the needle (fitted to a syringe) through the skin directly into the lumen of the vein. A gentle traction on the plunger of the syringe permits the barrel to fill. To avoid a hematoma, light digital pressure must be applied at the point of entry of the needle as it is being removed. Much more pressure is added after the needle is out, and the grip above the knee is loosened at the same time. Under these conditions, the minute wound seals itself very quickly. The blood is gently expelled from the syringe, immediately, into a calcium-free glass centrifuge tube, and the blood-stained syringe is placed in distilled water to prevent sticking.

In the dog, the peak of the blood calcium rise occurs from sixteen to eighteen hours after subcutaneous injection of the parathyroid extract. Therefore, the animals must be injected so that the final bleeding can be taken care of conveniently at the proper time, that is, during working hours. It has been customary, here, to make the parathormone injections between 4 and 5 o'clock in the afternoon of the day on which the normal blood was taken, and the final bleeding on the following morning between 8 and 9 o'clock.

All administrations of parathormone are made subcutaneously on the side of the dog. These injections are placed well toward the ventral surface in the loose tissue found there. The skin at the point of entry of the needle is pinched between the thumb and forefinger, just as the needle is withdrawn, to prevent leakage, and the injected area is massaged thoroughly to insure dispersion of the dose.

*Glassware*—All the glassware used in the test must be calcium-free. It is allowed to stand in hot cleaning fluid (100 gm of potassium dichromate to 4 liters of commercial sulphuric acid) for one hour, then passed in succession through 7 changes of distilled water. For the purpose of drying, it is placed in an inverted position on filter paper for about three hours. The drying process is completed in an electric oven at 100 to 110° C.

The steel needles, graduated syringes, and rubber stoppers are not placed in the acid bath but are thoroughly brushed and cleaned in distilled water, and then, with the exception of the stoppers, are taken through the same procedure as the rest of the glassware. The stoppers are dried at room temperature on filter paper.

*Calcium Determination*—The Tisdall modification of the Kramer-Tisdall method<sup>5</sup> is employed for the quantitative determination of the blood serum calcium. Clean glassware, and practice and care in technique are the chief requirements for making this an accurate test. For the practical purpose of standardizing parathormone, this method suffices.

The gasometric calcium method of Van Slyke and Sendroy<sup>6</sup> and the colorimetric method of Roe and Kahn<sup>7</sup> may be more precise, but they are much more complex in manipulation.

*Calculation of Unitage*—Since the parathormone unit is defined as one one-hundredth of the amount required to raise the calcium level of a 20 kg dog, 5 mg per 100 c.c. of blood serum, the unitage calculation takes the following form:

$$\frac{\text{Group A Ca rise (mg per 100 c.c.)}}{\text{Parathormone dose (c.c. per 20 kg)}} = \text{mg Ca rise per c.c. parathormone}$$

One unit is responsible for 0.05 mg calcium rise per 100 c.c. of blood serum

$$\text{Then the } \frac{\text{mg Ca rise per c.c. parathormone}}{0.05 \text{ mg}} = \text{units per c.c. parathormone}$$

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## A NOTE ON THE LIEBERMANN-BURCHARD COLOR REACTION FOR CHOLESTEROL\*†

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IT IS now a common observation that the Liebermann-Burchard reaction for cholesterol under uncontrolled conditions is not a reliable one. Temperature, intensity of light and time are all factors which tend to affect this reaction (Myers and Wardell,<sup>1</sup> Cornell,<sup>2</sup> Elman and Taussig,<sup>3</sup> Mattice†) Consequently, we are frequently confronted with the difficulty of obtaining duplicate results to check a single sample of blood. The literature abounds with suggestions for eliminating such discrepancies but we have found none of these efficacious.

During the course of our preliminary study of methods for the determination of cholesterol in the blood, we experienced great difficulty in getting checks closer than 10 per cent, irrespective of the colorimetric method employed. We finally decided to use Sackett's modification of Bloor's method<sup>4</sup> because of its simplicity, reliability, and inexpensiveness. However, our early determinations with this method showed a discrepancy of about 10 per cent between duplicate samples. In order to find the cause of this variation, we studied every aspect of the method. The procedure consists in adding 0.2 cc of plasma (or whole blood) to a mixture of 9 cc of alcohol and 3 cc of ether and subsequently evaporating the alcohol-ether extract to dryness. The cholesterol in the residue is then extracted with 5 cc of chloroform. To this is added 0.1 cc of sulphuric acid and 2 cc of acetic anhydride and a green color results (Liebermann-Burchard reaction).

A common argument against this method is the fact that a brown discoloration is frequently produced on rapid evaporation of the alcohol-ether extract

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†It has been observed independently by Miss M. R. Mattice in the Biochemical Laboratory of this institution that the color produced in the Liebermann-Burchard reaction may vary from a blue to a greenish yellow depending in a large measure on the temperature at which the reaction is allowed to take place.

This is eliminated completely if the evaporation is allowed to take place slowly in an incubator at 37° C (Rabinowitch<sup>5</sup>)

The two chloroform extractions as suggested by Sackett are absolutely complete, further extractions of the alcohol-ether residue with chloroform are unnecessary

We then considered the effect of temperature, and here we found the source of our discrepancies. The simple procedure of cooling the solutions after adding the sulphuric acid and the acetic anhydride in cold running tap water, as suggested by Myers and Wardell,<sup>1</sup> was tried on many occasions but the checks obtained were poor. It was felt that more uniform low temperatures were desirable and, indeed, necessary. In order to fix the temperature at which the color was produced at a low level and at the same time keep the solutions in darkness, we placed our extracts in a refrigerator which maintained a constant temperature of 5° C. This procedure immediately gave us more uniform results. Still better duplicate determinations were obtained when the Liebermann-Burchard reaction was allowed to take place for five minutes in a dark closet and then another ten minutes in the refrigerator. Controlled experiments with varying amounts of standard solutions showed that the maximum color is obtained under

TABLE I  
TYPICAL RESULTS OBTAINED WITH THE LIEBERMANN BURCHARD REACTION

SAMPLE	REACTION AT ROOM TEMPERATURE		SAMPLE	REACTION AT 5° C	
	CHOLESTEROL MG PER CENT	MEAN DIFFER- ENCE PER CENT		CHOLESTEROL MG PER CENT	MEAN DIFFER- ENCE PER CENT
1 a	213	10.2	6 a	231.5	0
b	192.2		b	231.5	
2 a	187.8	13	7 a	141.0	1
b	164.8		b	139.5	
3 a	243.5	8	8 a	161.0	0.9
b	264.0		b	159.5	
4 a	273.7	10.4	9 a	216.7	0
b	246.7		b	216.7	
5 a	307.5	15.2	10 a	213.0	0
b	264.0		b	213.0	

these conditions. The solutions were removed from the refrigerator one at a time and read against the standard cholesterol solution prepared under the same conditions. When readings are made with artificial light (shades of green are better differentiated with artificial light), it is advisable not to read more than six solutions against the same standard, since heat and light alter the green to a greenish yellow fairly rapidly and thus tend to vitiate the results.

Table I indicates the typical results obtained when the color reaction occurs at room temperature and those obtained with the procedure outlined above. The simple expediency of allowing the reaction to take place at a temperature of



refrigeration ( $5^{\circ}\text{C}$ ) has given us results which check within 2 per cent for a single specimen. Only rarely do we find discrepancies greater than this.

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### A METHOD OF MEASURING CHOLESTEROL\*

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#### INTRODUCTION

THE final estimation of cholesterol by the colorimetric method is difficult. This is so because the green color developed for the reading on the colorimeter is a factor which varies independently of the quantity of cholesterol. The standard if permitted to age before using in a determination will possess a green color which is different from that produced in a freshly prepared standard. The green color which develops in either the standard or the unknown cholesterol solution has only a fleeting maximum color intensity which rapidly fades. This change is due to the conversion of the green color to yellow producing many yellowish shades of green which are entirely unreliable for matching.

It was felt that possibly the yellow color could be utilized as a method of measurement of the cholesterol, in as much as such a color was very easy to match. This yellow color, it was found, could be very easily produced if the cholesterol solutions made up as for a green comparison were permitted to remain in a dark cabinet at about  $20^{\circ}\text{C}$  for a period of twelve to twenty-four hours. Moreover, the yellow color was found to be far more stable than the green.

#### METHOD

The procedure is a modified Myers-Wardell<sup>1</sup>. One cubic centimeter of whole blood which had been collected in a sterile oxalate tube (oxalate equivalent to 2 c.c. of 1 per cent solution) was placed in an extraction thimble in which had been placed loosely packed fat-free filter paper. This blood was dried for three hours at a temperature of  $70$  to  $80^{\circ}\text{C}$  and then extracted continuously with 20 c.c. of chloroform at  $70$  to  $80^{\circ}\text{C}$  for two and one-half hours. The chloroform extract was made up to 25 c.c. at  $20^{\circ}\text{C}$ . Into each of two tubes was placed 0.1 c.c. of concentrated sulphuric acid and 2 c.c. of acetic anhydride. Into one tube was placed 5 c.c. of the

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TABLE II

SPECIMEN	READING OF STANDARD	READING OF UNKNOWN IN		DIFFERENCE	MG CHOLESTEROL PER 100 C C BLOOD		DIFFERENCE
		GREEN	YELLOW		IN		
					GREEN	YELLOW	
1	15	19	21	2	157	143	14
2	15	24	26	2	125	115	10
3	15	20	22	2	150	136	14
4	15	23	25	2	130	120	10
5	15	28	30	2	108	100	8
6	15	32	30	2	93	100	7
7	15	24	26	2	124	115	9
8	15	25	23	2	120	130	10
9	15	24	22	2	125	136	11
10	10	27	30	3	74	66	8
11	15	27	29	2	111	113	2
12	15	19	18	1	157	166	9
13	15	25	24	1	120	125	5
14	15	31	32	1	99	96	3
15	15	20	24	4	150	125	25
16	15	22	25	3	136	120	16
17	15	27	28	1	111	107	4
18	15	30	30	0	100	100	0
19	15	33	30	3	90	100	10
20	15	33	30	3	90	100	10
21	15	28	31	3	108	99	9
22	15	32	30	2	93	100	7
23	15	24	27	3	124	111	113
24	15	25	22	3	120	136	16
25	15	24	20	4	125	150	25
26	10	30	27	3	74	66	8
27	15	27	29	2	111	103	8
28	15	19	18	1	157	166	9
29	15	19	22	3	157	136	21
30	15	24	26	2	124	115	9
31	15	20	22	2	150	136	14
32	15	23	24	1	130	124	6
33	15	13	15	2	230	200	30
34	15	28	25	3	107	120	13
35	15	21	18	3	142	166	24
36	10	27	26	1	74	76	2
37	15	24	22	2	125	136	11
38	15	20	19	1	150	157	7
39	15	21	18	3	142	166	24
40	15	17	20	3	176	150	26
41	15	20	17	3	150	176	26
42	15	16	14	2	187	294	27

evident from this table that in pure cholesterol solutions the estimation by the use of the yellow color is much more accurate than by the use of the green color

Table II presents the results of the cholesterol estimation of blood specimens by both the green and yellow colors. Here, too, it is evident that there is a difference in the amount of the estimated cholesterol when the two colors are used.

Inasmuch as the yellow color resulted in the more accurate cholesterol estimations in the pure cholesterol solutions and inasmuch as the cholesterol estimations in the blood specimens by the yellow color were different from those obtained by the green color in both positive and negative directions, it is felt that the measurement of cholesterol by means of the yellow color is more accurate than when the green color is used.

## SUMMARY

A method of preparing cholesterol for estimation is described. This method is more satisfactory and more accurate than the original colorimetric method of estimation.

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## A SIMPLE METHOD FOR THE PREPARATION OF HEMATOPORPHYRIN\*†

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## INTRODUCTION

THERE are a number of well characterized diseases in which the group of substances named porphyrins seems to play a definite rôle. Hematoporphyrin is a member of this group. In the last few years new light has been thrown on the clinical picture by the experimental data which has been accumulated by investigations on the physiologic, pathologic, photosensitizing, acute intoxication and chronic intoxication behavior of hematoporphyrin. Drs. Cornbleet, Bachem, and Reed of the staff of the University of Illinois College of Medicine have been studying the biologic behavior of this substance. They were handicapped because of the lack of reasonable quantities of pure hematoporphyrin.

## PROBLEM

It was this difficulty which suggested a study of the available methods. They were found satisfactory for the most part but so time consuming, that for the preparation of relative large quantities of pure hematoporphyrin they were impractical. By modification and combination of some of the desirable steps in the older methods, it was possible to easily prepare relatively large quantities of pure neutral hematoporphyrin, free from protein, iron, and other undesirable by-products yet possessing all the physical and chemical, fluorescent and spectroscopic properties attributed to it.

## SOURCE

The most common source of hematoporphyrin is the blood. It has been prepared from all types of blood pigments and its derivatives as hemoglobin, oxyhemoglobin, carbonmonoxide hemoglobin, reduced hemoglobin, methemoglobin, hemin, hematin and hemaehomogen.

Hematoporphyrin has been found in the contents of the stomach and intestinal tract after the introduction of acids in the stomach.

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Gaird in 1892-1900<sup>1</sup> found hematoporphyrin in the urines of normal and pathologic patients afflicted with cirrhosis of the liver and obstipation

MacMunn in 1885<sup>2</sup> and Hammaisten in 1891<sup>3</sup> found hematoporphyrin in urines from patients who were poisoned with sulphonal and trional

H Fischer and Kogl in 1923<sup>4</sup> prepared hematoporphyrin from highly colored feathers

H Fischer and Zeile in 1929<sup>5</sup> synthesized hematoporphyrin in small quantities

#### METHOD OF PREPARATION

In this method the source of hematoporphyrin was oxalated blood. Equal parts of oxalated blood and concentrated sulphuric acid were stirred until well mixed, and then allowed to cool. Four volumes of distilled water were added to one volume of this solution, which was then further allowed to cool. The solution was then filtered. The filtrate, a clear red solution, was neutralized with concentrated sodium hydroxide solution. Hematoporphyrin in the form of a brown amorphous precipitate appeared at or slightly to the acid side of the neutral point. The mixture was then filtered. The precipitate and filter paper were dissolved in just a sufficient amount of concentrated sulphuric acid and cooled. This acid solution was diluted with distilled water as above, one part solution to four water, further allowed to cool, and then neutralized as above with concentrated sodium hydroxide solution. Again the same brownish precipitate appeared. The mixture was then tested for the presence of protein by the Biuret test on a small portion of the filtrate. A positive test indicated the necessity of a repetition of the solution and precipitation process. The process was repeated as often as necessary to free the solution from protein. When the mixture was protein free, the precipitate from the last neutralization was allowed to settle out. The supernatant fluid was removed and replaced with distilled water. The mixture was then dialyzed in a collodion bag against distilled water until sulphate and non free, and dried at room temperature.

#### RESULTS

*a Physical Properties*—Hematoporphyrin prepared by this method was in precipitate form, dark brown, flaky, and amorphous. When dry has a dark red, almost black, color, with a definite violet shimmer and is friable.

*b Chemical Properties*—The product was easily soluble in concentrated sulphuric acid, hydrochloric acid, nitric acid, potassium, sodium, ammonium hydroxide, dilute acetic acid and sodium carbonate. Slightly soluble in dilute sulphuric acid, glacial acetic acid, methyl alcohol, benzol, nitrobenzol, petroleum ether, acidified ethyl alcohol. Insoluble in water, 96 per cent ethyl alcohol, ether, chloroform. In acid or alkaline solution it can be precipitated at neutrality point with neutral salts as magnesium sulphate, ammonium sulphate, and potassium chloride. A test for iron was negative. At 100° C it is destroyed giving a pyrol odor.

*c Fluorescence*—When ultra-violet light is passed through a chamber containing a suspension in distilled water of this preparation it gives a brown fluorescence, in acid solution a dark red, and in alkaline solution a purple red.

*d Spectrum Absorption*—The material was examined spectroscopically with a Hilger Spectroscope. The solutions contained 0.05 per cent of hematoporphyrin,

and the acid used was sulphuric acid and the alkali, sodium hydroxide. The bands obtained were found similar to those attributed to acid and alkaline hematoporphyrin. No evidence has been found in the literature for neutral absorption bands.

TABLE I

<i>Alkaline</i>				
	I	II	III	IV
$\frac{1}{2}$ cm	620 uu—weak	566 uu—weak	539 uu—weak	517 uu—strong
1 cm	615 uu	576 uu	540 uu	519 uu
<i>Acid</i>				
	I	III		
$\frac{1}{2}$ cm	594 uu—narrow	550 uu—broad	Absorption Beginning at 490 uu	
1 cm	594 uu	(570) 550 uu		
<i>Neutral</i>				
	I	II		
$\frac{1}{2}$ cm	536 uu—very broad	500 510 uu		
1 cm	566 uu	510 uu		

## DISCUSSION

The method described is a composite of the desirable features from the following methods:

Laidlaw<sup>6</sup> was among the first to use whole blood and concentrated sulphuric acid. The resulting mixture was allowed to stand from three to four days, then extracted with alcohol and evaporated to dryness.

Hoppe-Seyler<sup>7</sup> was the earliest to describe a method and he named the material hematoporphyrin. His method uses hemin which necessitated the production of hemin.

Eschbaum<sup>8</sup> modified the Hoppe Seyler method in that he used whole blood and concentrated sulphuric acid. The resulting mass was extracted with absolute alcohol and neutralized with an alcoholic solution of sodium hydroxide.

The Nencki Sieber method<sup>9</sup> is by far the most elaborate. Their method as modified by Willstätter and Fischer<sup>10</sup> is as follows: Hemin is treated with a solution of hydrogen bromide in glacial acetic acid, then allowed to stand for five days. The solution is then diluted with water, filtered, and neutralized with sodium hydroxide. The product is freed of impurities by washing the precipitate with a solution of dilute acetic acid until free of chloride. Dhere-Sobolewski<sup>11</sup> added dialysis of the solution after precipitation with sodium hydroxide.

The methods mentioned are all time consuming and involve many technical procedures. With the exception of the Nencki Sieber method the product obtained is not a pure hematoporphyrin. All give a poor yield.

## SUMMARY

The method described is simple and rapid because it is applied to whole blood. The quantity of concentrated sulphuric acid is important since higher concentrations of acid will tend to form compounds other than hematoporphyrin. Lower concentrations tend to allow precipitation of the pigment. This method completely removes the protein. Dialysis is of very definite value because the iron salts and sodium sulphite dialyze through while pigment remains in the bag. Finally the product is a pure neutral hematoporphyrin.

## CONCLUSIONS

- 1 A simple method for the preparation of hematoporphyrin is described
- 2 The product obtained by the method is a neutral hematoporphyrin
- 3 The properties of the product are those of hematoporphyrin
- 4 It is free from iron, the sulphate ion and protein

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## A TURTLE BOARD WHICH SERVES SEVERAL LABORATORY PURPOSES\*

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THE turtle board described below, devised in the Laboratory of Pharmacology of the George Washington University School of Medicine, has been found to be a valuable addition to the locker list and a description was thought advisable

The board consists of a base (*A*) and two uprights (*B1, B2*), and closely resembles the inverted form of the ordinary type of operating board used for mammals. The base (*A*) and uprights (*B1, B2*) are made of dressed white pine, six-eighths of an inch in thickness. The base (*A*) is seven and one-half inches wide and twelve inches long. The uprights (*B1, B2*) are seven inches long and one and six-eighths inches in height, and are fastened to the base (*A*) by a copper screw in the center and nails near the ends. The most satisfactory positions of

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the uprights, leg and head fasteners and holes for the head cord are shown in Fig 1

When the turtle is used, the animal is placed in an inverted position in the uprights ( $B1, B2$ ) with its head toward the end of the base ( $A$ ), the legs are

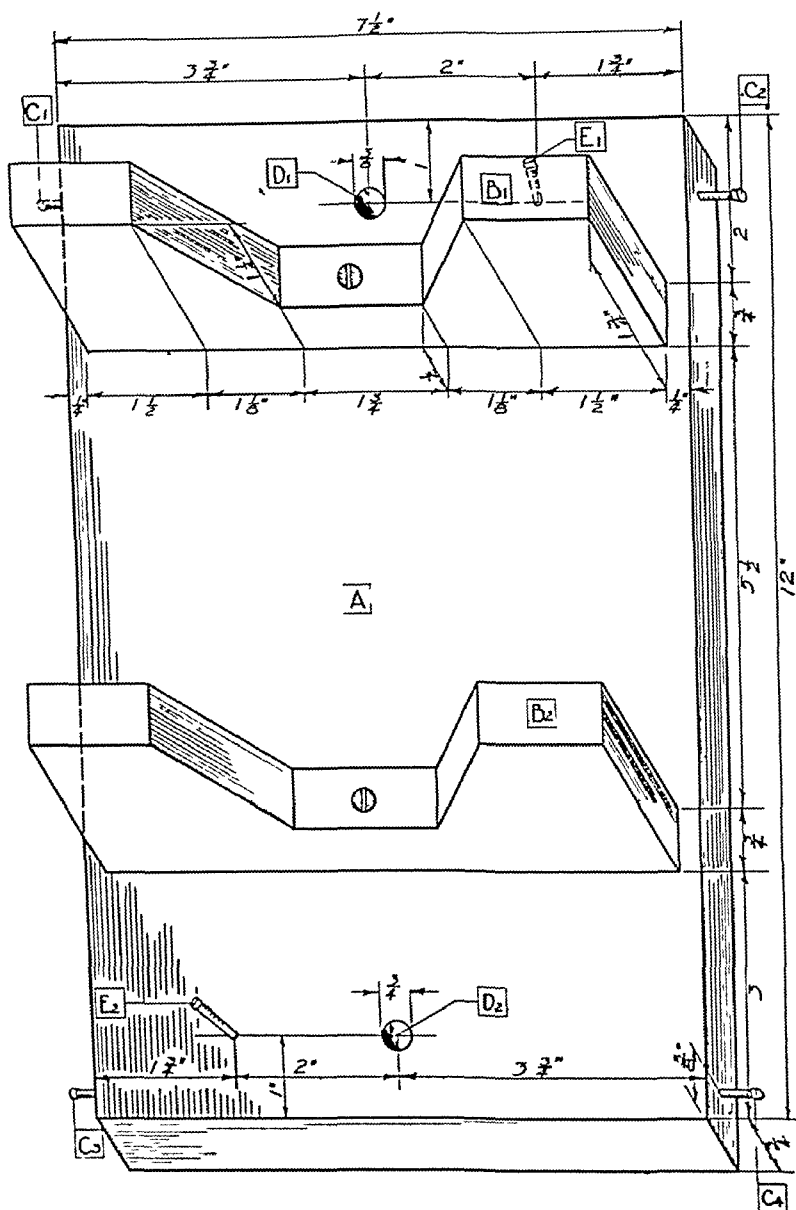


Fig. 1—Drawn to scale.  $A$  base  $B1, B2$  uprights  $C1, C2, C3, C4$  leg fasteners.  $D1, D2$ , holes for head cords  $E1, E2$  head fasteners.

firmly tied to the leg fasteners ( $C1, C2, C3, C4$ ), a heavy cord is tied around the neck with the knot at the base of the head, and the free end of the cord is carried over and under the end of the base, up through the hole ( $D1$  or  $D2$ ), pulled taut, and tied to the fastener ( $E1$ , or  $E2$ )

This board is adaptable without change for all the usual sizes of turtles used in the laboratory. Its small size avoids interference with stands for holding heart levers, etc., and its relatively light weight does not preclude its use for perfusion work, when clamped to an upright stand. The board, when resting on the uprights, may be used as a kymograph stand, and also as a frog board, if holes for leg fasteners are drilled in the base.

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## A DIFFERENTIAL STAIN FOR DRIED, UNFIXED VAGINAL SMEARS\*

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W. KENNETH CUYLER, M. A., CLEVELAND, OHIO

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IN STUDYING the action of various hormones on the genitourinary organs, the white rat has been used. During these studies vaginal smears were made daily, for consecutive weeks, on many animals. Five smears were made on one microscopic slide in order that a complete estrus cycle might be studied on a single mount. The smears were made in half-inch areas, leaving ample room at one end for labeling.

Since it was impractical to stain daily the fresh smears as they were added to the slide, it was necessary to use a stain which would give good results with dried, unfixed smears. It was found that various dyes stained well, but that the cells could not be differentiated in smears made during the characteristic phases of the reproductive cycle. Hence, it became necessary to employ a stain which would react with old and unfixed material, and also differentiate the various kinds of cells found in the smears.

The stain which finally was found to meet all requirements was Harris' hematoxylin followed later by a counterstain consisting of indigo-carmin and yellow eosin. The counterstain was made from indigo-carmin (Gruber's), 0.25 gm., eosin Y (Coleman and Bell), 1 gm., and distilled water, 99.00 cc. A small lump of thymol was added to prevent the growth of mold. No fixing agent was required before staining.

The staining procedure was as follows. The slides were (1) immersed in alcohol (95 per cent), for three minutes, then (2) in distilled water for one minute, (3) immersed in Harris' hematoxylin (or Delafield's hematoxylin) for twenty minutes, (4) washed in tap water for two minutes, then (5) in alkaline alcohol (70 per cent alcohol made by using a 4 per cent solution of aqua ammonia instead of distilled water), (6) soaked in the counterstain (indigo-carmin and eosin), eight hours or overnight, (7) washed quickly in tap water, (8) dehydrated quickly in absolute alcohol, then (9) cleared in xylol, and finally (10) mounted in balsam.

In the application of this technique, the cells could be identified as follows. The nuclei of the epithelial cells were gray, while the cytoplasm was lavender. The cornified cells were bright pink. The leucocytes had dark nuclei while the cytoplasm was clear. The red blood cells were pink.

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\*From the Cleveland Clinic Foundation.

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Vaginal smears made in this way may be handled the same as paraffin sections in regard to destaining after the hematoxylin. No destaining is required, however. In fact, it was found that the stain was intensified both by the tap water used to wash off the hematoxylin and by alkaline alcohol.

This process of staining vaginal smears requires considerable time, which, however, the excellent results in differentiation would seem to justify. It is possible to hasten the procedure somewhat by warming the staining solutions, but this is not to be recommended, because when this is done the slides are likely to become overstained.

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## CASE OF DYSENTERY (FLEXNER) TREATED BY BACTERIOPHAGE\*

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GEORGE S. DAVENPORT, M.D., AND SIGURD W. JOHNSEN, M.D., PASSAIC, N. J.

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ONE of us (S. W. J.) obtained from the Bacteriological Laboratory of Columbia University, New York Post-Graduate School, an agar slant culture of *Bacilli dysenteriae* (Flexner) and an effective bacteriophage, for experimental work. The *Bacilli dysenteriae* had been isolated from a case of acute dysentery at the Post-Graduate Hospital. The bacteriophage had been isolated from sewage, and produced a complete lysis of the organisms in four hours. Through careless manipulation S. W. J. became infected and developed an acute dysentery.

Following is the history. On Friday night November 6, 1931, three days after obtaining the culture, marked restlessness and abdominal distress developed. Several chills and an attack of vomiting followed. A large stool was then evacuated, with severe griping. Three additional watery stools were evacuated by morning. Temperature 101° F, pulse 90.

A suspicion of accidental infection by *Bacilli dysenteriae* (Flexner), prompted a stool examination and preparation of a bacteriophage. Five cubic centimeters of fresh bacteriophage which caused complete lysis *in vitro* of the organisms, in four hours was taken immediately, by mouth.

Dr. Davenport was then called to attend the case. Following are his findings:

Young man, aged thirty five years, weight 145 pounds, appears acutely ill, temperature 101° F, pulse 90. Chest and heart normal. Abdomen is slightly distended, with generalized tenderness. Urine examination is normal. The patient was put to bed. 8 P.M. Temperature was 102° F, pulse 94. Twelve watery stools, no blood. Slight dehydration.

Sunday 8 A.M. Temperature 101° F, pulse 90. Marked prostration. Ten stools evacuated during night, dark brown in color, moderate amount of mucus.

Five cubic centimeters of bacteriophage, in water, given by mouth. Buttermilk and tea given *ad lib*.

12 noon. Temperature 99° F, pulse 80, 5 c.c. of bacteriophage in a little water, given by mouth.

3 P.M. Temperature 99° F, pulse 80. Patient comfortable. Three stools during day. Patient sleeping at intervals.

Monday. Report of stool examination shows *Bacilli dysenteriae* (Flexner) present. Specific bacteriophage absent from stool.

Patient had six stools in past twenty four hours. Large amount of mucus, no free blood. Marked prostration is present. Temperature 98° F, pulse 80.

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\*Received for publication December 7, 1931.

Tuesday Patient up and around, but is still weak Stool examination was negative for dysentery bacilli Positive for bacteriophage

Complete lysis of organisms in four hours Urine examination is normal Weight 134 pounds

Friday Stool examination was negative for *Bacilli dysenteriae* Positive bacteriophage was present Complete lysis in four hours

One week later stool examination was negative for dysentery bacilli and negative also for bacteriophage

#### COMMENT

The effectiveness of specific bacteriophage therapy in this case is very striking

The disappearance of *Bacillus dysenteriae* (Flexner) from the stool was rapid The course of the infection was shortened and recovery was prompt

The absence of a potent bacteriophage at the onset, and its presence for five days after the last oral dose was taken, is also significant

49 PASSAIC AVENUE.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**MENINGITIS**, *Staphylococcus*, Treatment With Specific Bacteriophage, Schelss, R. A. *Am J Dis Child* 44: 813, 1932

A case is reported with recovery after intraspinal use of staphylococcus bacteriophage.

A review of the literature of staphylococcal meningitis is given, a classification of clinical types offered and a résumé of therapeutic measures and their efficacy made.

The literature of bacteriophage, to date, is covered, and evidence of the applicability of this agent to other staphylococcal infections is quoted.

A report on the first instance of the introduction of bacteriophage into the spinal canal is given, with comment on the resultant recovery in a true case of *Staphylococcus aureus* meningitis.

**ERYTHROCYTES**, Diameter of In Premature Infants, Van Creveld, S. *Am J Dis Child* 44: 701, 1932

By means of the method of Price Jones the changes in the size of the erythrocytes during the first months after birth were investigated in a large number of full term and premature infants. One of the chief reasons for this study was the fact that directly after birth the blood is influenced by different factors which in themselves are able to change the size of the erythrocytes in a definite way. These factors do not act with the same intensity in full term as in premature children. During the first two weeks after birth the Price Jones curves of full term and premature infants showed little difference. Whereas after that period the mean diameter in full term children did not change very much, that in premature children decreased considerably, also the curve showed a different course from that in full term children. The significance of the established differences and their relation to the newer conceptions of the cause of hemolysis after birth, to the anemia in premature children and to the physiologic loss of weight after birth are discussed in extenso.

The clinical significance of the anisocytosis and of the mean diameter of the erythrocytes in the analysis of anemias in the early months of life is explained by means of some examples (cases of icterus neonatorum gravis and congenital anemia).

**BLOOD** Clinical Value of Uncorrected Color Index and of Cell Size in Pernicious Anemia, Goldhammer, S. M. *Am J M Sc* 184: 645, 1932

The uncorrected color index represents the actual hemoglobin per cent reading (100 per cent as normal), over the percentage of red blood cells (5,000,000 being equivalent to 100 per cent), disregarding such factors as sex and the various hemoglobin standards.

Because of the wide range of normal red blood cell counts, 5,000,000 cells per cmm may be used as an average arbitrary standard. From 14 to 16 gm of hemoglobin per 100 cc of blood are suggested as a standard of normal.

The average and serial color indexes in uncomplicated cases of pernicious anemia are above 1 before treatment, those of the male series are higher than those of the female.

Regardless of type of effective treatment, the influence on the color index is the same, the average and serial color indexes remain above 1 for about 6 weeks after treatment is started, then become less than 1.

In untreated and uncomplicated cases the lower the initial red blood cell count the higher the average color index. As the red blood cell count approaches the arbitrary normal following adequate treatment, the color index tends to approximate unity. Within the range of 4,000,000 to 5,000,000 red blood cells per cmm a level is reached at which the color index became 1 or less.

A single color index determination is not always diagnostic in cases of pernicious anemia, as individual readings may be above or below 1, but during relapse the average color index is always above 1 unless some complication is present

The color index may be influenced by such complications as hemorrhage, chronic infections, glandular dystrophies and food deficiencies

The increase in the percentage of red blood cells larger than 7.5 micra in early relapse, regardless of the severity of the anemia, may be used as a factor differentiating pernicious anemia from secondary anemia

In untreated cases of pernicious anemia a high color index is always associated with a marked increase in the percentage of cells larger than 7.5 micra

The presence of increased numbers of large cells in a blood film, regardless of the red blood cell count, is one of the earliest and most constant findings of the blood in beginning relapse in pernicious anemia

RENAL FUNCTION, An Improved Concentration Test, Lashmet, F. H., and Newburgh, L. H.  
J. A. M. A. 99 1396, 1932

The following instructions are furnished the patient

Instructions for Renal Function Test

- 1 Date
  - 1 At 10 P. M. stop all fluid and food, except special diet until noon (38 hours)
- 2 Date
  - 1 At 8 A. M. empty bladder and discard urine
  - 2 Eat special diet only today
  - 3 Collect all urine from 8 A. M. today until 8 A. M. in bottle 1 Finish specimen promptly at 8 A. M.
- 3 Date
  - 1 No breakfast today
  - 2 Collect urine at 10 A. M. in bottle 2
  - 3 Collect urine at 12 noon in bottle 3

Collect urine in dry bottles. Keep bottles in a cool place and keep well stoppered

The special diet referred to is shown in the table

Menu of the Diet

	Foods	Grams	Approximate Measure
Breakfast,	Corn flakes	15	$\frac{1}{2}$ cup
	Bread (toast)	60	2 slices, $\frac{3}{8}$ inch
	Butter	20	2 squares or 1 level tablespoon
	Cream 40%	50	$\frac{1}{4}$ cup
	Sugar	13	1 tablespoonful
Lunch,	Beef steak	100	4 by 4 by $\frac{1}{2}$ inch
	Potato (baked)	80	1 about $2\frac{1}{2}$ inches diameter
	Crackers	16	4
	Butter	20	2 squares, or 1 level tablespoon
	Dates	70	10
Dinner,	Potato (baked)	80	1 as above
	Lettuce	100	$\frac{1}{2}$ head
	Crackers	16	4
	Butter	20	2 squares, or 1 level tablespoon
	Dates	30	4
	Peaches (canned—no juice)	85	1 half

In addition to foregoing, 1 gm. of sodium chloride (table salt) or  $\frac{1}{4}$  level teaspoonful. No other salt is to be added to cooking or otherwise

The diet above furnishes 700 gm. of available water in 24 hours and contains protein 40 gm., fat 104 gm., carbohydrate 204 gm. equivalent to 1900 calories. It contains 6.536 gm.

of nitrogen, 9.1 gm of inorganic solids, 3.286 gm of chlorine, and an excess of available base equivalent to 18 c.c. of normal solution

**The Test** All three specimens are tested quantitatively for albumin and their specific gravity recorded. For every 1 per cent of albumin 0.003 is subtracted from the observed specific gravity

**Results** Under definitely controlled conditions, normal kidneys are able to concentrate the urine to a specific gravity between 1.029 and 1.032, while diseased kidneys cannot. The lower the concentrating ability is, the greater the renal damage.

When determined under the conditions described, the specific gravity of the urine detects lowered kidney function before this fact is discernible by either the phenolsulphonphthalein test or determination of the blood nonprotein nitrogen.

#### Granulopenia and Agranulocytic Angina, Harkins, H. J. A. M. A. 99 1132, 1932

Reports of thirty six cases of recurrent granulopenia are collected from the literature.

Seventeen cases of granulopenia have been observed in the University of Chicago Hospitals. Eight of these seem to be primary granulopenia and in four more than one attack occurred.

The etiology of primary granulopenia is as follows:

- (a) The oral lesions and sepsis are secondary to the granulopenia.
- (b) The primary granulopenia may be due to some endogenous factor such as allergy, endocrine disturbance or congenital deficiency of the bone marrow.
- (c) On the other hand, the causative factor may be an exogenous agent, acting either on a normal person or on a person with a special susceptibility. Unknown organisms, *B. influenzae*, and chemical poisons are to be considered.

(d) The causative agent acts chiefly on the bone marrow.

The following conclusions are made concerning the treatment of primary granulopenia:

(a) Oral antiseptics merely delays the entrance of malignant organisms into the system. Conversely, attempts to restore the white cell count to normal will not save the patient's life if sepsis is too advanced.

(b) Blood transfusion and roentgen rays are of no proved value.

(c) Chemical agents intended to stimulate the bone marrow are in general ineffective. Some evidence has collected to indicate that nucleotide is of value in primary granulopenia. Nucleotide did not affect the course of the granulopenia in acute benzene poisoning in rabbits.

#### BLOOD PLATELETS. A Method for The Simultaneous Enumeration of Reticulocytes and, Dameshek, W. Arch. Int. Med. 50 579, 1932

This is an indirect method in which the diluting fluid is not only isotonic but contains brilliant cresyl blue for staining purposes and sodium citrate for use as an anticoagulant. This solution, which is a modification of the one devised by Buckman and Hallisey for counting the platelets directly, contains the following ingredients:

	gm. or c.c.
Brilliant cresyl blue	0.15
Sodium citrate	0.40
Sucrose	8.00
Water	100.00

The cane sugar and the sodium citrate are dissolved in distilled water to which is then added the brilliant cresyl blue. The resulting solution is mixed well and filtered. Three drops of a solution of formaldehyde (1:10) U.S.P., are added as a preservative.

This solution keeps well in a cold place, but it should be filtered every three or four weeks. It has the following advantages: (1) The stained platelets are easily seen. (2) The stained network and granules of the reticulocytes are well brought out. (3) The anticoagulant prevents clumping of the platelets. (4) The isotonicity of the solution aids in the proper separation and distribution of the blood cells.

**Method**—One of the fingers is well cleaned with alcohol or acetone and then dried. A puncture wound is made. The first drop of blood is discarded. A fairly large drop (about 3 mm. in di-

ameter) of the staining solution is placed over the puncture wound, and the finger is gently squeezed so that a small amount of blood wells up into the drop of staining solution. The correct amount of solution to be placed on the finger and the proportion of blood to be squeezed into it can be learned only with experience. The proportion of blood to stain should be small (about 1:5) so that a well spread preparation, not overcrowded with red blood cells, is obtained. The mixture of blood and stain is immediately transferred to a cover slip, which is then dropped on a slide. Cleanliness of glassware is essential.

The preparation is examined under oil immersion lens, preferably after from fifteen to forty five minutes to permit complete staining of the platelets and reticulocytes. Counts may be made at any time within two hours. Counts made up to four hours, if the preparations are ringed with petrolatum, are also accurate. Permanent preparations cannot be made with this method. Blood platelets, even those measuring only 1 micron or less in diameter, are easily seen as highly refractile opalescent bodies taking a pale bluish stain. Reticulocytes are well stained, even the slightest degree of granulation and reticulation being easily seen. The rapid motion of some of the reticulocyte granules can easily be followed. White blood cells, which also take the stain, may easily be recognized and counted if desired.

One thousand red blood cells are counted (the microscopic field being cut down if desired by the insertion in the eyepiece of an appropriately perforated paper disk), and the number of platelets and reticulocytes seen during this enumeration is recorded. The number of reticulocytes is expressed as a percentage of the total number of red blood cells. The absolute number of platelets is obtained by (1) performing a count of the red blood cells, and (2) solving the following equation, red blood count 1,000 platelets per cubic millimeter platelets counted. This is most conveniently done by multiplying the first four figures of the red blood cell count by the number of platelets seen in counting 1,000 red blood cells. Thus, if in counting 1,000 red blood cells, 20 reticulocytes and 200 platelets are seen, and the red blood cell count is 3,000,000 per cubic millimeter, the percentage of reticulocytes is 2, and platelet count is 3,000 times 200, or 600,000 per cubic millimeter.

Normally, there is slight variation in the size and shape of the blood platelets.

The normal blood platelet count with this method in men ranges from 500,000 to 900,000 per cubic millimeter. The normal count in women is made uncertain by the complicating presence of the menstrual cycle. The intermenstrual range is from 400,000 to 800,000 per cubic millimeter.

Various procedures to check the accuracy of the method were undertaken. The experimental error is probably not greater than 70,000 per cubic millimeter when the erythrocyte count is normal and not more than 10,000 per cubic millimeter when the erythrocyte and blood platelet counts are very low.

#### **DIAZO REACTION** A Modified, in the Diagnosis of Typhoid Fever, Symons, P. H. S. A. Med J 6 594, 1932

The method following is said to be more satisfactory and to give more reliable results than those heretofore used.

**Stock Solutions**—A Dissolve 0.9 gram of sulphaniic acid in water, add 9 c.c. of 37 per cent HCl, make volume up to 100 c.c. with distilled water.

B Make up 5 grams of sodium nitrite to 100 c.c. with water.

C A 1:1 per cent aqueous solution of pure anhydrous sodium carbonate or a 3 per cent solution of crystalline sodium carbonate is used as the alkali.

**Preparation of Reagent**—In a beaker containing an ice water mixture place an empty test tube and a graduated cylinder containing 41 c.c. of distilled water.

Into the test tube pipette 15 c.c. of A and 15 c.c. of B, both cold, leave for five minutes, add 6 c.c. of C, again leave for five minutes, then pour the contents of the test tube into the water in the graduated cylinder, in which it has meantime been chilled by immersion in the ice water mixture. Fifty cubic centimeters of reagent are thus obtained. The reagent is stable for at least twenty four hours in the cold.

As the reagent decomposes on warming, evolving nitrogen, the evolution of a copious volume of gas while preparing it indicates that the temperature is too high and the reagent useless. The

reagent should also be quite colorless. When ice is not available, good results may often be obtained by substituting cold water, particularly if the solutions A and B be mixed slowly. The use of freezing mixtures might be considered.

**The Test**—Mix 2 c.c. of the reagent with 5 c.c. of C. Within a minute add from a dropping pipette one drop of the urine to be tested, so that it falls slowly through the solution. Observe the color development of the drop of urine against a white background.

A positive reaction is shown by the immediate development of a red or orange red color, which sometimes disappears very rapidly. Practically all urines give a yellow color which slowly intensifies, this may be neglected.

It was not considered worth while to estimate the strength of the reaction with quantitative accuracy, so that the following conventions were adopted.

One drop of urine dropped slowly through the reagent gives

- (1) A yellow color, intensifying slowly = negative (-)
- (2) A brownish color = doubtful (?)
- (3) A faint pink = doubtful (?)
- (4) A definite pink = positive (+)
- (5) Red = moderately strong positive (++)
- (6) Deep red = strong positive (+++)

Urines for the test should be fresh and free from obvious bile, stale normal urines tend to give a brownish coloration, probably due to free ammonia. Production of color on mixing the reagent with solution C is usually due to a dirty tube.

#### **B. PROTEUS** The Isolation of Organisms In The Presence Of, Fry, R. M. Brit. J. Exper. Med. 13, 456, 1932

The method depends on the fact that in the depths of a solid medium *B. proteus* grows in discrete colonies. The swab or other material from which a culture is to be made is spread on blood agar, and melted agar at about 45° C. is then poured over the surface of the plate to a depth of 2 or 3 mm. and allowed to set. After incubation it will be found that any colonies of *B. proteus* growing between the two layers of agar show no tendency to spread, and colonies of other organisms can be easily picked out from among them. It generally happens, however, that some of the proteus spreads round the edge of the agar on to the upper surface, and this must be killed before any deep colonies are picked out. A satisfactory method is by flooding the surface of the plate with saturated mercuric chloride solution for about thirty seconds. This is washed off with tap water, and some of the surface growth of proteus is scraped off with the end of a microscope slide to enable the deep colonies to be examined. These can then be picked out and subcultured.

If the colonies are very crowded, and it is thought that the one picked out may be contaminated by a neighboring colony of *B. proteus*, it is advisable to cover the subculture with a layer of agar in the same way. By this means, repeated more than once if necessary, it is possible to get a pure culture of most organisms, no matter how thick the contamination may be.

#### **TISSUE STAIN** Mallory's Connective Tissue Stain Following Hematoxylin, Weiss, S. Stain Techn. 8, 131, 1932

Slightly overstain in Mayer's hematoxylin (50 gm. potassium alum and 0.2 gm. sodium iodate added to 1 liter 0.1 per cent aqueous hematoxylin). Wash, and stain 30 seconds to 1 minute in 0.04 per cent aqueous acid fuchsin. Stain 4 minutes in 0.5 gm. anilin blue and 2 gm. orange G dissolved in 100 c.c. of 1 per cent aqueous phosphomolybdic acid. Pass through 95 per cent alcohol to absolute, clear in alcohol and mount in balsam.

#### **TYPHOID FEVER, H and O Agglutination As An Aid In The Diagnosis Of,** Dulaney, A. D., Wikle, W. T., and Trigg, R. Am. J. Pub. Health, 22, 1033, 1932

From a study of 161 serums from 41 cases of typhoid, 30 cases of nontyphoid febrile diseases and 90 vaccinated (anti-typhoid) individuals, the H and O titers being determined in each case, the authors conclude that

- 1 Both H and O agglutinins are produced in typhoid fever
- 2 O agglutination in high titers (1:500 or greater) is very suggestive of typhoid infection
- 3 Lower titers do not rule out O agglutination due to related infections or vaccination

**AMEBIASIS, Craig Complement Fixation Test For In Chronic Ulcerative Colitis, Kiefer, E D**  
*Am J M Sc* 183 624, 1932

That it is extremely difficult to differentiate between chronic ulcerative colitis and chronic amebic colitis has been pointed out and discussed

The result of the Craig complement fixation test for infection with *Endamoeba histolytica* when applied to patients with chronic ulcerative colitis is reported showing that 15 out of 19 cases gave a strongly positive reaction

No conclusions as to the significance of this finding are drawn, but it is suggested that chronic ulcerative colitis may be a pyogenic infection of the colon superimposed upon an original amebic ulceration

Abstracts of the case histories of the 19 cases tested are reported

**MELANOMA Simple Technique for the Dopa Reaction, Ludlow, G T, and Blackberg, S L**  
*Am J Path* 8 491, 1932

The reagents required are a stock solution of dopa and the Sorensen buffers

The Stock Dopa Solution is a 1:1000 solution of 3,4-dioxyphenylalanine (abbreviated to dopa) in distilled water (When ordering, specify "for Bloch's dopa reaction" Supplied by the American branch of Hoffmann-LaRoche, Nutley, New Jersey, at 95 cents per gram With the minute quantity used, the cost of staining a dozen or more sections is less than 2 cents)

Dissolve 0.3 gm of dopa powder in 300 cc of cold distilled water Keep well corked in the refrigerator, where it will remain good for many weeks The solution is usable as long as it is colorless or only slightly tinged with red Darker red solutions should be rejected, they oxidize too quickly and overstain the sections

#### THE BUFFERS

Dissolve 11 gm of disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$  plus  $2\text{H}_2\text{O}$ ) in 1000 cc of distilled water

Dissolve 9 gm of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) in 1000 cc of distilled water Both of these buffers are kept in the refrigerator

Just before cutting the sections, buffer to 7.4 by adding 2 cc of the potassium phosphate and 6 cc of sodium phosphate buffer to 25 cc of the stock dopa solution For a small batch of a dozen sections we use 15 cc of the buffered solution, but we prepare double the quantity immediately required in order to have enough to renew solution in half an hour Return the stock dopa solution and the surplus of the buffered solution to the refrigerator immediately, at room temperature the stock solution soon oxidizes and turns red, the buffered solution tends to turn brown

At a given temperature, the speed of the reaction is determined by the  $\text{P}_\text{H}$  At 7.4 the reaction will be finished in four or five hours at 37° C

#### TEMPERATURE AND TIME

The dish of dopa containing the sections is put in the incubator at 37° C for about half an hour Then the fluid is replaced by fresh solution, which in the meantime has been kept cold in the refrigerator For this renewal of the solution there is a reason Some tissues are sufficiently acid to lower the  $\text{P}_\text{H}$  below 7.0, in which event the fluid remains red and the cells do not oxidize dopa to melanin They remain colorless On the other hand, tissue that has been in formalin, especially neutralized formalin, hastens the oxidation, darkens the fluid prematurely and easily overstains Since it is impossible to foresee the presence of these disturbing factors, and since water cannot be used to wash them out, it is a routine practice to change the dopa The first dopa washes out any objectionable substances and the reaction proceeds unhindered in the fresh solution At times sections of the rectum and colon are so acid that two changes of dopa are required before the red of the acid solution changed to sepia brown of a correct reaction Under these circumstances a liberal quantity of dopa solution should be used

Having replaced the first dopa with fresh solution, the reaction is inspected every half hour In two or three hours the fluid turns reddish, then sepia brown The appearance of the sepia tint signals the end of the reaction At this point a section is rinsed and examined under the microscope



## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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### The Cardiac Output of Man in Health and Disease

**A**N inclusive review of historical investigations, a detailed presentation of the author's acetylene method of determination, and a comprehensive discussion of the clinical and experimental significance of this method of study. For further remarks see editorial in this number.

### Physiological Chemistry

**M**ATHEWS' Physiological Chemistry is so well known as a standard textbook that it requires no introduction. A volume which retains this distinction for fifteen years and passes through five editions must necessarily both fill the need for which it was written and avoid the extremes and superlatives that occasionally detract from the value of a book written by one who is an enthusiast in some particular branch of his chosen field.

There has been no significant change in the arrangement or manner of presentation in the latest edition, although the recent advances in the field of physiological chemistry have been incorporated and the volume is entirely up to date.

### A System of Clinical Medicine

**T**HIS is not so much a textbook of medicine as a treatise on physical diagnosis and differential diagnosis, combined. It opens with general instructions on the taking of a history and the making of a physical examination and then takes up the various regions of the body, classifying the illnesses appertaining thereto on the basis primarily of symptoms. Following a discussion of symptoms there is a detailed discussion of physical signs and any laboratory procedures that may be indicated, and then a presentation of differential diagnosis. Prognosis and treatment are lightly touched upon.

The fact that this volume has gone through eight editions indicates its worth. It would find its chief usefulness with students of medicine and with general practitioners who have but few of the more highly technical volumes to refer to.

### The Clinical Interpretation of Blood Examinations

**T**HE intention of this volume is indicated sufficiently in its title. The clinician or the general physician having received a brief report from the laboratory concerning some particular test, what is to be the correct interpretation as regards this particular patient?

- \*The Cardiac Output of Man in Health and Disease. By Arthur Grollman, PH.D. M.D. Associate Professor of Physiology in the Medical School of the Johns Hopkins University. Cloth. Pages 72. Springfield, Ill. Charles C. Thomas, 1932.
- \*Physiological Chemistry. A Textbook and Manual for Students. By Albert P. Mathews, PH.D. Carnegie Professor of Biochemistry, The University of Cincinnati. Fifth Edition. Illustrated. Cloth. Pages 1233. William Wood & Company, New York, 1930.
- \*A System of Clinical Medicine. Dealing With the Diagnosis, Prognosis and Treatment of Disease for Students and Practitioners. By Thomas Dixon Savill, M.D. London. Fifth Edition. Cloth. Pages 1019. William Wood & Company, New York, 1930.
- The Clinical Interpretation of Blood Examinations. By Robert A. Kilduffe, A.B. M.D. F.A.S.C.P. Director Laboratories, Atlantic City Hospital. Consulting Serologist, Betty Buchrach Home for Crippled Children, Atlantic City. N. J. City Bacteriologist, Atlantic City. N. J. Pathologist, Atlantic County Tuberculosis Hospital, Northfield. N. J. Consulting Pathologist, Jewish Seaside Home, Atlantic City. N. J. Etc. Octavo. 629 pages. Illustrated. Cloth. Lea & Febiger, Philadelphia, 1932.

The present volume is the outgrowth of two smaller volumes by the same author, "The Clinical Interpretation of the Wassermann Reaction," and "The Clinical Interpretation of Blood Chemistry." Both of these smaller volumes found an immediate use and the author was stimulated to round out the subject by covering all phases of any form of examination of the blood that is done in the clinical laboratory. His effort has been most successful in its comprehensiveness and in its simplicity.

The literature has been very thoroughly covered and where opinions differ as to interpretation, these differences are recorded. Rarely used tests are included with those which are frequently employed and the most recent are also included. The volume will appeal to the internist equally as much as to the general practitioner.

### Conquering Arthritis

**T**HIS volume, written for the layman, particularly the person suffering from arthritis, serves the purpose first of acquainting him with the nature of his malady and second, of enabling him to develop a healthy optimism concerning his disability. The work presents nothing particularly new from a research point of view but otherwise should be of interest not only to the layman with or without arthritis, but also to the physician who has made no special study of the disease but who wishes to inform himself on the most recent developments in classification and treatment.

### The Doctor and His Investments†

**T**HIS is really a worth while book. One has not read far into it before one becomes convinced of the sincerity of the writer and of the fact that the book is in no sense a stock promotion scheme. Mr. Rukeyser has contributed widely to The Journal of the American Medical Association's medical economics, Dental Survey, several of the prominent New York papers and is associate in journalism at Columbia. The type of advice that he gives is sound, if only one would follow it when it actually comes to making investments. Possibly, for the younger physician, the more important part of the volume is the negative part, that is, a discussion of who is economically not in a position to invest in stock. The writer emphasizes especially that one should cover his future by adequate life insurance before considering any other investment. He is not at all certain that home owning is a good thing unless one has a large family. He is still more dubious about owning stock in medical art buildings. The discussion of stocks and bonds is very much along the same line that one would find in any other introductory discussion of the subject, but throughout the author applies the facts to the needs of the physician.

The volume can be most highly recommended.

\*Conquering Arthritis By H. M. Margolis M.D. Cloth Pages 192 The Macmillan Company New York 1931

†The Doctor and His Investments Financial Policy and Technique for the Physician By Merrylye Stanley Rukeyser B.Lit. M.A. Financial Editor Medical Economics and Dental Survey Cloth Pages 330 P. Blakiston's Son & Co. Inc. Philadelphia.

# *The Journal of Laboratory and Clinical Medicine*

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## EDITORIAL

### Cardiac Output as a Functional Test

THE popularization of studies of functional pathology has resulted in the elaboration of very adequate methods for the study of the functional capacity of the kidneys, the thyroid gland, the internal secretion of the pancreas, the stomach and to a certain extent the liver. Progress has not been as rapid in the development of methods for study of circulatory function. Attempts have been made to assay the function of the circulatory and respiratory systems by vital capacity studies such as those elaborated by Dreyer<sup>1</sup> and by Peabody and Wentworth,<sup>2</sup> or the elaborate system devised by Frost,<sup>3</sup> and by study of the response of the pulse and blood pressure to exertion, and the breath holding, expiratory force, and expiratory fatigue test of Flack,<sup>4</sup> but all of these have been found to have only limited value, the conclusion usually being reached that the patient's subjective symptoms still remain the best measure of circulatory efficiency. And yet it is

at once obvious that such criteria will show evidence of functional impairment only after such impairment has progressed to a relatively pronounced degree

Much can be learned of the conduction function within the heart by the electrocardiogram but the need of a more direct and definite measure of cardiac function has been obvious. Methods of estimating the output of the heart have been devised and improved over years until they have been so simplified that we may look toward the perfection of this test to a stage where it can be used simply and easily as an office routine. If, as stated by Grollman, the cardiac output per minute, ratioed to body surface area, is a constant, it is readily understandable that this ratio, termed the "cardiac index," may assume a clinical value comparable to that of the metabolic rate determination.

Early studies of cardiac output were necessarily crude and were made by such methods as measuring the size of the ventricles in the cadaver, measuring output in lower animals and calculating the probable proportionate output in man, measuring the rate and volume of blood flow in an artery and calculating therefrom that in the aorta, measuring the appearance time of potassium ferriocyanide in the vein of one extremity after its injection into the vein of the opposite extremity, plethysmographic studies, and other ingenious indirect methods.<sup>3</sup>

Fick in 1870 elaborated a concept which has since been called the Fick Principle which with adaptations, modifications and improvements has been the basis of most of the newer methods and simplifications of this study.

One can determine the amount of oxygen consumed by a body per minute time (as in the familiar metabolism test). One can determine the volume of oxygen in 100 c.c. of arterial blood and that in 100 c.c. of mixed venous blood by direct measurement. The difference between these two latter figures represents the amount of oxygen given up to the tissues by each 100 c.c. of blood. The total oxygen consumption per minute divided by the number of volumes given up to the tissues by each 100 c.c. of blood indicates the number of 100 c.c. volumes of blood which must have passed through the heart and been distributed to the tissues per minute time. Fick also pointed out that the same information could be obtained by knowledge of the carbon dioxide content of arterial and venous blood and the carbon dioxide output per unit time through the lungs. If arterial blood contains 18 volumes per 100 c.c. of oxygen and venous blood from the right side of the heart contains 12 volumes, then every 100 c.c. of blood gave up 6 volumes of oxygen to the tissues. If the total oxygen consumption of the individual is 60 c.c. per minute, it is obvious that ten 100 c.c. volumes each giving up 6 c.c. are necessary to distribute the 60 c.c. to the tissues in one minute's time. The cardiac output per minute is then 1000 c.c.

Obviously, the necessity of obtaining blood by puncture of an artery and directly from the right ventricle or from the vena cava prevent clinical application of this method. Gradually other methods have been elaborated based upon the Fick Principle, first using inert gases such as nitrogen and later, foreign gases. The method recommended by Grollman<sup>6</sup> involves the use of acetylene gas.

If a gas is in contact with a liquid it will become dissolved in and diffused through that liquid. The greater the concentration of the gas above the liquid the more will be dissolved in the liquid. The greater the volume of liquid the

less will be dissolved per unit volume. If one is breathing acetylene gas, this gas is in contact with a liquid (blood) in the lungs. If one knows the concentration of the acetylene gas in the air of the lung and its concentration in the blood per unit volume (in equilibrium with the acetylene in the air of the lungs) and if one knows the solubility of the gas in blood, one can compute the total volume of blood required to make that concentration in the blood. The tension and rate of absorption of acetylene are determined and from this the volume of blood flowing through the lungs per unit time is calculated. This of course corresponds with the volume flowing through the heart per unit time except in certain rare congenital anomalies.

It is not necessary to determine the concentration of acetylene in the blood. The volume of liquid in a gas-liquid system regulates the rate of absorption. If one knows the solubility of the gas in the liquid and its rate of absorption one can compute the volume of the liquid. In the case under consideration the volume of the liquid is the amount of blood passing through the lungs per unit time. One liter of blood at body temperature dissolves 740 c.c. of acetylene when the tension of this gas is 760 millimeters mercury. In the test, therefore, the patient rebreathes in a closed lung-bag system, a mixture of oxygen and acetylene. The acetylene concentration in this closed system is determined at the beginning and end of the experiment. The difference in these two concentrations divided by the time gives the rate of absorption. The solubility being known, the volume of fluid to which the gas was exposed may be calculated, and this is the cardiac output per unit time. Since the experiment involves respiratory activities which modify the normal circulation, certain corrections are made on the basis of simultaneous determinations of oxygen consumption in the lung-bag system and the determination of oxygen consumption per unit time as in the ordinary metabolism test.

Thus we see that a calculation of the cardiac output can be done simply, by methods of gas analysis, with certain corrections analogous to the corrections in the determination of oxygen consumption in metabolism tests. If, at rest, or under basal conditions the cardiac output is a constant, proportional to the surface area (the cardiac index), a method is at once available for study of the cardiac output in health and in the various diseases. The cardiac output of normal individuals per square meter of body surface is 2.2 plus or minus 0.3 liters per minute.

Grollman devotes considerable attention in his monograph to the results of clinical studies of cardiac output and to its possible clinical significance. For example, the intake of large amounts of fluid increases the cardiac output, thereby entailing more work on the part of the heart. In a heart already damaged by myocarditis this increased strain might work disadvantageously in certain cases of fever in which the usual procedure is the forcing of fluid. A knowledge of the cardiac output is of distinct importance in diseases of the heart and circulation but often desirable also in conditions in which the circulation is only secondarily affected. The moot question concerning the desirability of routine digitalization in pneumonia, previously discussed in these columns, may receive further elucidation by this method of study. As shown by Grollman, by Harold J. Stew-

ait and by others, a dilated heart when digitalized, returns to approximately normal size with a resultant increased cardiac output. A normal heart on the other hand when digitalized, contracts to less than normal size, with resultant decreased output. This would appear to be a potent argument against routine digitalization except in the presence of an obviously diseased, and especially a dilated heart.

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W T V

# The Journal of Laboratory and Clinical Medicine

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No 4

## CLINICAL AND EXPERIMENTAL

### PATHOGENIC YEASTS AND YEAST-LIKE ORGANISMS

REPORT OF A CASE IN MINNESOTA SIMULATING COCCIDIOIDAL GRANULOMA\*

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#### YEASTS AND YEAST-LIKE FUNGI

MANY varieties of unicellular fungi called yeasts and similar forms producing mycelium, exist in nature. We are directly concerned with these fungi in this paper, since some of them possess pathogenic properties and may constitute the exciting agents of granulomatous and purulent inflammations of man.

True yeasts are defined by mycologists as unicellular fungi, spherical or oval, reproducing by budding or fission, and by sexual sporulation (ascospores), and usually inducing alcoholic fermentation in solutions of sugar. Occasionally, under special conditions, rudimentary mycelium may be evolved.

Yeast-like, that is, unicellular budding forms, are not alone distinctive of true yeasts. They also occur in all of the great classes of fungi, as a temporary mode of growth, in response to environment. The unicellular growth may in fact temporarily supplant the mycelial form in fungi inherently capable of forming mycelium. When fungi assume such unicellular formations they are referred to as yeast-like, but the term as used in a restricted sense designates those fungi which exhibit the yeast-like growth as a common feature of their development.

We may presume, as Hennen did, that the yeasts have been derived from more complex fungi, representing those which have permanently lost the power to form true mycelium. Their relationship to the more complex mycelial fungi is shown by their occasional attempts to form rudimentary mycelium, by their similarity to the yeast-like cells of certain mycelial fungi, and by their methods of sexual sporulation, in all, affording rather certain knowledge of their ancestry. Thus, it has been possible to classify side by side the unicellular yeasts and mycelial-producing fungi,

\*1 from The Mayo Clinic and The Mayo Foundation  
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the essential difference being that mycelium is formed in one and only single budding cells in the other

The precise classification for true yeasts and the related mycelium-producing fungi is schematically shown (adapted from Castellani<sup>1</sup>) in Table I

TABLE I  
YEASTS AND ENDOMYCETES

ENDOMYCETES (TRUE FUNGI)			
CLASS	ORDER	FAMILY	GENUS
		Saccharomycetaceae (Rees) No definite mycelium	Saccharomyces (Meyer) True yeasts Unicellular budding forms, occasionally rudimentary mycelium, reproducing by smooth ascospores, producing alcoholic fermentation or sugar solutions
Ascomycetes Ascospores (endospores) present, mycelium septate when present	Saccharomycetales Asci not gathered into definite perithecia	Endomycetaceae (Rees) Mycelium present	Endomyces (Rees) Yeast like and mycelium formations, reproducing by ascospores, external spores and spores situated inside the mycelial tubes

TABLE II  
YEAST-LIKE ORGANISMS OF FUNGI IMPERFECTI

ENDOMYCETES (TRUE FUNGI)				
CLASS	ORDER	SUBORDER	FAMILY	GENUS
			Cryptococcaceae (Kützing) Hyphae and spores alike, that, is yeast like	Torul (Persoon) Usually nonpathogenic and not producing alcoholic fermentation
Fungi imperfecti Ascospores absent, mycelium septate when present	Thallosporales Reproduction by thallospores (thallus or body secondarily adapted to reproduction) mycelial hyphae more than 1 micron in diameter	Blastosporineae Reproduction by blastospores (a budding thallospore)	Oosporiaceae (Saccardo) Mycelial hyphae present, spores typically in chains	Cryptococcus (Kützing) Pathogenic, with well developed double contour
				Oidium (Link) Hyphae, long branched, terminating in chains of spores, gas not formed in carbohydrate
				Monilia (Persoon) In situ budding forms and mycelium, in cultures, mostly budding, some filaments, dextrose fermented with gas



As the yeasts may be interpreted as having been derived from more complex fungi, so also are less complex types probably derived from them, and the closely related Endomyces. These more simple types are regarded as imperfect fungi but they usually possess a prototype among those fungi which have perfect classifications. The imperfection consists in an unknown sexual sporulating stage. The imperfect fungi, a more or less heterogenous group, many of which are yeast-like, have been classified together under the name of fungi imperfecti. In referring to them Henriec remarked that the imperfection of the organisms is not entirely the result of an imperfect cycle, but in part, at least, due to our inadequate knowledge

SYSTEMATIC RELATIONSHIPS OF THE YEASTS

ASCOMYCETES                      FUNGI IMPERFECTI

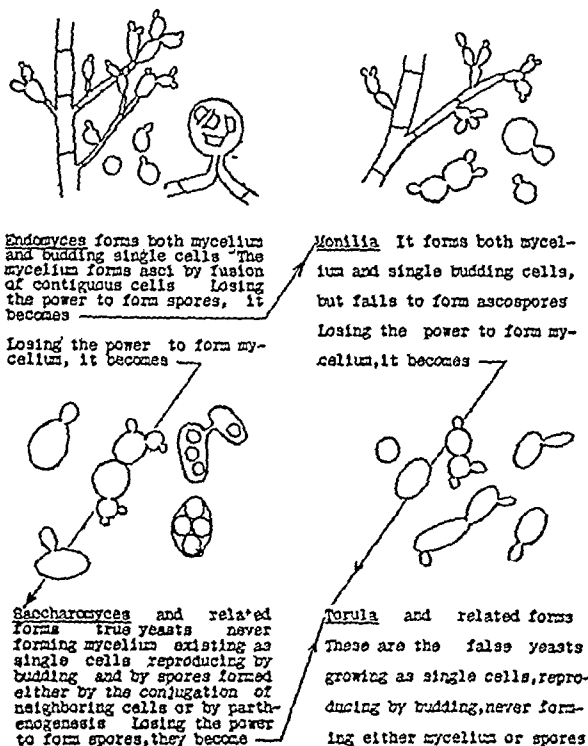


Fig 1—Systematic relationships of the yeasts

of them. It is thus evident that their present classification is a means toward an end and not final.

Fungi imperfecti are of several orders but the organisms pertinent to this consideration are classified in the order Thallosporales. A schematic illustration of their classification (adapted from Castellani<sup>4</sup>) is given in Table II.

Henrici schematically shows the relation which exists between *Saccharomyces* and *Endomyces*, *Monilia* and *Torula* (Fig 1<sup>o</sup>).

According to Guilhaumon and Timmer, yeasts were first described by Leewenhock, Mitscherlich, Cagniard Latour, Schwann and Kützing, about 1825, demon-

\*Reprinted by permission from *Molds, Yeasts and Actinomyces* by Arthur T. Henrici, published by John Wiley and Sons, Inc.

strated that yeasts of beer and wine were composed of cells which multiplied by budding. Endospores in yeasts were first observed by Schwann in 1839. He proved that they could be freed by rupturing the wall of the cell. Although yeasts had been closely allied with our knowledge of fermentation, their true significance dates from Pasteur's experiments in 1859. Hansen's description of their life cycle, their systematic relationship and classification, has been generally accepted. The discovery of zymase by Buchner, by which our knowledge of yeast nutrition and the mechanism of alcoholic fermentation has been considerably advanced, also has been fundamental.

Yeast-like fungi have been known since Link's description of *Oidium lactis* in 1809. Ricketts gave the characteristics of this genus apparently as defined by Link as follows: "A genus of fungi, having an elementary organization many species living as moulds. They have more or less ramifying and segmented filaments, which produce at their extremities short cells, very often rounded. These cells, which are united end to end, rosary-like, detach themselves when mature."

#### PATHOGENIC YEAST AND YEAST-LIKE ORGANISMS

About 1840, Robin and others, established *Oidium albicans* as the cause of thrush and other systemic lesions of man. Its relationship to human disease is well reviewed in Ricketts' monograph. The present tendency among mycologists is to regard the causative organisms of thrush as belonging to the genus *Monilia*. Castellani differentiated the two genera by gas fermentation reactions in dextrose. *Monilia* causes marked fermentation, *Oidium* little, if any. Henni differentiated on the basis of forming free cells, in *Oidium* they arise by disarticulation of the mycelium, and in *Monilia* by budding from the mycelium. Castellani, however, believes that the clinical syndrome of thrush may result from a variety of infecting organisms, of the genera *Oidium*, *Monilia*, *Endomyces*, and *Saccharomyces*. The *Oidium* (*Monilia*), it appears, was the first of the yeasts or yeast-like organisms to be associated with disease of human beings.

The pathogenicity of yeasts and yeast-like forms for experimental animals, as demonstrated by Popoff, Rabinowitsch, Sanfelice,<sup>32, 33, 34</sup> Roncali, Weis and others, in 1872 and later, was the next advance in our knowledge that these fungi have pathogenic properties. Their description as the "parasites of cancer," the consideration of which occupied the attention of investigators<sup>29, 30, 31, 32, 33, 34</sup> in the latter part of the nineteenth century, is of historic significance only, since the idea has long been abandoned.<sup>9, 22</sup>

The association of true yeasts as the cause of disease of man is credited to Busse<sup>2, 3</sup> in 1894. In Busse's case, the patient was a woman, aged thirty-one years. Multiple, disseminate, superficial and deep subcutaneous abscesses developed secondary to a periosteal lesion of the tibia, which resembled a softened giant-cell sarcoma. At necropsy systemic abscesses were also found. In the original "tumor," in the pus from the abscesses, and in the blood, Busse found numerous, doubly contoured, rounded or ovoid bodies which were classified by cultural methods as yeasts in the genus *Saccharomyces*. He named the organism *Saccharomyces hominis* and the disease *saccharomycosis hominis*. Using material from this case, Buschke concurred in most essentials, independently, with Busse's observations.

Curtis<sup>6, 7</sup> in 1895 in France, published a second case of true yeast infection,

although there seems to be more doubt concerning the nature of the organism in this case. Curtis' patient presented a "myxomatous tumor" of the thigh. From the so called tumor, organisms similar to those described by Busse were obtained, and although a cure had apparently been effected by surgical removal of the growth, Greenfield stated that this patient died ten months later of meningitis (possibly saccharomycetic).

There has been much controversy about these and similar cases of yeast infection, since there has been difficulty in accurately evaluating the original classifications. Stoddard and Cuttler and Greenfield classified the Busse-Buschke organism as *Saccharomyces*, while Castellani<sup>4</sup> and Vuillemin regarded it as *Cryptococcus* (a genus created by Vuillemin to accommodate the pathogenic *Toxula*-false yeasts). Greenfield and Castellani classified the Curtis organism as *Saccharomyces*, and Stoddard and Cuttler left it unclassified. In any event, one must conclude that infections in man, due to true yeasts, are comparatively rare.

The stimulus to other investigators to establish yeasts as the parasites of malignant tumors was provided by Busse's suggestion, in his first report, that the tibial lesion which his patient exhibited resembled a softened giant-cell sarcoma, and by Curtis' report, which described a myxomatous tumor, both apparently having yeasts as etiologic factors.

In 1896, Gilchrist<sup>10</sup> reported a case of scrofuloderma on the back of a man's hand, in which large numbers of rounded, doubly contoured, refractive bodies, measuring 10 to 16 microns in diameter, with numerous budding forms present, were observed. The case antedated Busse's report by six months, but since cultural studies were not obtainable, the identity of this organism was not disclosed.

Gilchrist and Stokes, in 1896,<sup>11</sup> presented a second case of a purely cutaneous disease termed by them pseudolupus vulgaris, in the tissues of which organisms similar to those previously described by Gilchrist and corresponding to those of Busse, were revealed. In cultures the organism appeared as a mycelial growth with distal and lateral cells or conidia in place of budding yeast-like cells, as had been observed in the tissues. For this reason and also because alcoholic fermentation did not result when grown in solutions of sugar, the organism was classified as an *Oidium*. In supplementary studies,<sup>12</sup> however, due to uncertainty in the classification of the yeasts, Gilchrist and Stokes reversed their former opinion and described the organism as a yeast, naming the parasite *Blastomyces dermatitidis* and the disease blastomycosis (the term *Blastomyces* was at that time quite widely used for certain yeasts). It was found that a number of "wild yeasts" do not ferment solutions of sugar and do form mycelium.

Weincke, from South America, in 1892, presented a case of doubtful mycosis fungoides in the lesions of which he observed protozoon-like forms. Rixford and Gilchrist in 1896 described similar organisms from two cases of a generalized chronic granulomatous disease, both of which had come from California. The parasites did not grow in artificial cultures so they concluded that they were dealing with a new form of protozoon infection resembling coccidiosis. Rixford and Gilchrist named the organisms *Coccidioides immitis* (or *pyogenes*), and the disease coccidioid or pseudotuberculosis. The pathologic anatomy of the lesions resembled closely that found in tuberculosis, fundamentally differing, however, in that pyogenic reactions were also frequent. The organisms were spherical unicellular

bodies, varying from 7 to 27 microns in diameter and consisting in their encapsulated condition of a thick, doubly contoured capsule enclosing a finely granular protoplasm. They developed by sporulation and often as many as 100 small spores or "sporozoites" were set free by the bursting of the capsule. They were occasionally found to be intracellular, but as a rule they were extracellular.

In 1900, Ophuls and Moffitt succeeded in demonstrating the true nature of the so called protozoon bodies. From a case similar to those described by Weiricke, and Rixford and Gilchrist, cultures of the protozoon bodies revealed them to be the fructification stage of a mold. The mold developed in cultures as a mycelium with aerial hyphae, and with bodies resembling oidia. In 1905, Ophuls after further study classified the fungus as a member of the genus *Oidium*, and proposed the designation *Oidium coccidioides* for the organism and *oidiomycosis* for the disease. He recognized the similarity between the coccidioidal organism and the organism of blastomycosis. He had been able to see the evolution of mycelium from the "protozoon-like" bodies when the latter were freed from the tissue in cultures. Wolbach in 1904 and MacNeal and Taylor in 1914 demonstrated its completed life cycle by showing that the mycelium, when introduced into susceptible animals, evolved the protozoon-like bodies.

The impression, generally held, that blastomycosis as described by Gilchrist and Stokes was only a cutaneous disease, was disproved by Walker and Montgomery in 1902 when they demonstrated a systemic disease, complicating blastomycosis of the skin, caused by organisms essentially identical with those that had been found in the cutaneous lesions.

In 1916, Stoddard and Cuttler reported a fourth type of infection due to yeast-like organisms. Their cases were peculiar in that the central nervous system was the principal site of involvement. The associated organisms were yeast-like in both the tissues and on cultures, evidencing growth by budding, without sporulating. Because of the yeast-like form which the organisms assumed under all conditions and due to failure to demonstrate reproduction by sporulation, they were designated as *Toxula*, with the specific name *Toxula histolytica*. The disease was called toxulosis. The organisms, as they appeared in the tissues, had the apparently distinctive characteristic of occurring in zooglyphic masses due to elaboration of a gelatinous matrix. The lesions appeared as either cystic or nodular areas in the brain or meninges, and were granulomatous in nature, sometimes with caseation necrosis. McGehee and Michelson have also recorded local infectious processes in other anatomic situations due to *Toxula*.

Although many other types of yeasts and yeast-like pathogens have been described, space does not permit their consideration here. Neither does it permit a complete consideration of their history.

#### COMMON FEATURES OF THE YEAST AND YEAST-LIKE PARASITES AND THEIR INFECTION

In agreement with the literature we have classified four rather well-defined diseases, each having a specific yeast or yeast-like organism as its etiology. On the other hand, it is only necessary casually to review the reports to be convinced that there exist many common features of morphology and growth. The organisms all maintain doubly contoured yeast-like morphology in the tissues and except in the case of *Coccidioides*, growth by budding is characteristic. In cultures some main-

tain the distinctly yeast-like morphology, others produce mycelium, but common to all are the yeast-like or oidial formations. Each of the diseases is characterized by chronic granulomatous reactions, more or less closely resembling tuberculosis, and also eliciting a variable pyogenic reaction. Each may have local cutaneous or subcutaneous manifestations or result in systemic infection. The portals of entry in each are similar: skins, lungs or other (unknown) internal organs permit the invasion. Referring to the clinical syndromes, Montgomery stated that "Coccidioidal granuloma, in spite of the literature to the contrary, may be indistinguishable clinically from certain cases of blastomycosis." The same statement could be broadened to include all of the diseases referred to in this paper. Any of the so-called distinctive clinical or pathologic characteristics of these diseases may be duplicated by others of the group. Castellani<sup>4</sup> remarked that each of these diseases is often referred to as blastomycosis, again suggesting the likeness of one to another.

This intimates that these diseases and their organisms are more closely related than might be assumed by the varied classifications encountered in their descriptions, certainly more closely related to one another than sporotrichosis or actinomycosis is related to them. For example, they exhibit a relationship comparable to that which exists between the various diseases caused by the parasites of the order Actinomycetales.

Variations might be explained on the basis of adaptation to environment such as is shown by Blastomyces (Gilchrist). Ricketts, Henner and others have shown that this parasite may assume three different types of growth in cultures, namely, yeast-like, oidium-like, and hyphomycetoid (Ricketts), or mealy, prickly and wooly (Henner), and that these characteristics are influenced by environment. All the different stages, Henner found, might occur in one and the same organism when subjected to changes which influence growth. In the tissues of the host some of the variations exhibited by the organisms could be similarly explained. The variations in the tissue reactions may be determined by the adaptive changes of the organisms or by other factors which influence the relationship between virulence of the organisms and resistance of the host.

Ricketts, in 1901, was the first to propose a simplified classification for the yeasts and yeast-like pathogens and the diseases resulting from them. He studied material and cultures from seventeen cases of the various diseases caused by yeast and yeast-like organisms. Fourteen cases were representative of cutaneous blastomycosis; the remaining three were of the Busse-Buschke case, the Curtis case, and the Ophuls-Moffitt case. Ricketts proposed to include all in the genus *Oidium*, as defined by Link and to name the diseases oidiomycosis. His conclusions have not been generally accepted because of his insistence on the genus *Oidium* for all. In placing the organisms in a common genus and relating the diseases to variations of an identical process, he proceeded farther than either contemporary or subsequent investigators have been willing to follow. To quote his conclusions in part: "The so-called protozoic disease of Posada, Weimcke and others, Busse's and Curtis' saccharomycosis hominis, and Gilchrist's blastomycetic dermatitis are various manifestations of the same disease. The organisms isolated from the various cases differ in minor respects among themselves but are so closely related morphologically as to justify their inclusion in a common genus, *Oidium*, they are

thus analogous in a pathogenic sense to the fungi which cause actinomycosis and to those causing trichophytosis "

#### CLASSIFICATION OF THE PATHOGENIC YEASTS AND YEAST-LIKE ORGANISMS

The organisms of Busse's and Curtis' cases and a few others, correspond to true yeasts. Their proper designation should be *Saccharomyces*, as originally proposed for them.

*Torula* of Stoddard and Cuttler are also probably in the present stage of knowledge, properly designated as such or as *Cryptococcus*. Characteristics which might serve better to classify them have not been described.

With *Blastomyces* the systematic position is not so clear. The organism is neither a true nor a false yeast, although it has certain yeast-like forms. Ricketts and Stoddard and Cuttler allocated it to the genus *Oidium*. Hennie also favored this view, using the designation *Oidium* for those forms which give rise to free cells by disarticulation of the mycelium as in *Oidium lactis*.

*Coccidioides*, because of its apparent reproduction by endosporeulation in the tissues and because of its cultural characteristics of mycelial growth, has been generally assigned to the Ascomycetes (family Endomycetaceae). Although its cultural characteristics support Ophuls' view that it is an *Oidium*, endogenous sporulation as it occurs in the tissues is not consistent with the genus *Oidium*, or other types of Fungi imperfecti. Endospores in such numbers, however, are not found in other species of the Endomycetaceae, the family to which this parasite has been provisionally assigned in present classifications<sup>14</sup>. Also opposed to the classification of *Coccidioides* as an Ascomycete is the fact that no trace of sexuality has been found in the development of the spores (Hennie).

These individual designations are presented with the understanding that the organisms usually offer sufficiently distinctive characteristics to classify as such, but also with the knowledge that they constitute a biologically unsettled group subject to mutations and variations among themselves, thus exhibiting varied growth potentials, each organism being fundamentally closely related as would be the case if each were derived from a common form. In place of allocating all of the organisms to the genus *Oidium* and designating all of the diseases as oidiomycosis, as Ricketts did to satisfy this relationship, it seems preferable to refer to them according to their predominating characteristics, using specific names for this predominance as we have done, but with an appreciation of their close relationships. We may illustrate this point of close relationship by referring back to the schema, as proposed by Hennie (Fig 1), substituting these pathogenic forms for the homologous types as they are visualized in the schema, when, for example, *Coccidioides* would occupy the position with *Endomyces*, *Saccharomyces* would occupy the position with other true yeasts, Gilchrist's blastomycete (under existing knowledge best regarded as an *Oidium*) would occupy the position with *Monilia*, and *Torula* would occupy the position with the homologous nonpathogenic *Torula*.

This approach of the subject does not offer a perfect solution but it serves a useful purpose, although admittedly temporizing until more certain knowledge is at hand concerning the life cycles of the organisms. It demonstrates a mutual relationship between the organisms and the diseases which they produce, and substitutes an understandable explanation where confusion had existed.

## COMMON FEATURES OF BLASTOMYCES AND COCCIDIODES, THEIR ULTIMATE CLASSIFICATION

Most of the difficulties have arisen over attempts to classify *Blastomyces* and *Coccidioides*. *Coccidioides* reproducing in the tissues by endogenous sporulation and not by budding is apparently distinctly different from *Blastomyces* wherein reproduction is assumed to occur only by budding. Some of the attempts which have been made to reconcile the systematic position of these two organisms to existing classifications, or which demonstrate their perfect classification will be cited.

Castellani has taken the position that the so called spores of *Coccidioides* are in reality not spores, but represent exaggerations of the granules and spherules common to both *Blastomyces* and *Coccidioides*. The sporulation stage of both, according to this opinion, is still to be found. On this assumption he reclassified the organisms of both diseases in a common genus *Blastomycoides* (of the Fungi imperfecti, Oosporaceae). So far there appears no confirmation of this opinion.

If the organisms of both diseases were of the genus *Oidium*, as Ophuls and Ricketts held, the problem of classification would be reduced. This, however, so far as *Coccidioides* is concerned, seems untenable for reasons already given.

Assuming that, as is quite likely true, *Coccidioides* reproduces in the tissues by endospores, and may be classified as an Ascomycete of the family Endomycetaceae, the perfect classification for that organism has been attained. For *Blastomyces* the perfect classification is as yet problematic, but certain characteristics which have been reported from time to time tend toward ultimate classification of this fungus in a similar if not identical systematic position. The only distinctive classifiable feature which *Coccidioides* holds over *Blastomyces* is that in the former an endosporulating stage has been found. When a similar stage has been discovered for *Blastomyces*, this essential difference will be removed. Then possibly the two organisms will be found in the same or related genera.

A few observations have been made which tend toward solution of the problem in this way. Ricketts was the first to suggest ascospore production in the organisms of blastomycosis. He observed endocellular bodies in the protoplasm of budding cells in six cases of cutaneous blastomycosis (Cases 3, 6, 7, 8, 9, 10 of his monograph). He proposed that these inclusions might prove to be spores but at the same time commented that final evidence of this was wanting. LeCount's and Myers' case was more convincing, in which the same dual processes of reproduction, as Ricketts had described, were seen. Their descriptions also included the finding of empty spore cases, apparently left behind by rupture and discharge of the spores. Stober made similar observations in one case and also found bodies in one of his cultures resembling ascospores. Montgomery and Ormsby noted bodies which strongly suggested endospores in some organisms, but they were unable to observe their further development. Commenting on this, they stated "It may be that further study will remove the one fundamental difference between them (blastomycosis and coccidioidal granuloma), that is, the behavior of the organisms in tissue, and prove the conditions to be but varieties of the same process." Wade described a case which exhibited innumerable organisms of small size in the tissues of a case of blastomycosis, but he interpreted their formation to rapid budding of immaturely developed forms. He did not regard the presence of empty "spore sacs" as significant.

Mellon recorded the most direct evidence of sporulation by Blastomyces. He observed the formation of four-celled asci in cultures of the parasites of blastomycosis. On the basis of his observations he suggested that these organisms should be allocated among the Ascomycetes (Endomyces) rather than with the Oidia of Ricketts or the Cryptococci of Vuillemin. Confirmation of this finding is all that is needed for its general acceptance. Thus, the taxonomic position of these organisms would be established.

Vuillemin observed similar reproductive bodies in an organism isolated from a case of thrush, which led him to classify it in the class Ascomycetes as Endomyces albicans. This has been suggested as the perfect stage of Monilia albicans. Castellani also described Endomyces as the etiologic factor in thrush but believed such a finding is illustrative of plurality of etiologic factors rather than an indication of relationship between them.

With such data forming the basis of opinion, it may be anticipated that eventually the organism of thrush (Monilia), the organism of blastomycosis (Oidium), and the organism of coccidioidal granuloma (Coccidioides) will be classified side by side as Endomycetaceae, closely related to the true yeasts, which are in the neighboring family Saccharomycetaceae. The Torula, by more convincing evidence of their suspected close relationship to the true yeasts, would also be included with the Ascomycetes. Thus their complete life cycle would be demonstrated and the proposal for a simplified classification, of apparently related forms, would reach fulfillment in a perfect classification of each form.

In the case reported, the finding of apparent sporulating bodies and budding forms in the tissues supports the view that Blastomyces and Coccidioides are closely related forms.

#### REPORT OF CASE

A man, aged forty one years, was admitted to The Mayo Clinic March 14, 1931. He was born in Germany and immigrated to America at the age of twenty four. He settled on an Iowa farm and ten years later moved to southern Minnesota, where he also engaged in farming. He had not travelled elsewhere in the United States. About January 1, 1931, he slipped and fell backward, striking his head on a brace. Following this, he appeared to have lost strength and occasionally experienced periods of mild vertigo. February 15, he again struck his head while straightening up from a stooping position. This was followed by headache of frontal and occipital type, which persisted constantly, with periods of great severity. The headache was increased by standing and considerably eased when lying down. About March 1, he began to experience intermittent attacks of vomiting, and noticed a swelling on the inner side of his left leg. Otherwise his past history was essentially negative, except for occasional colds of the thorax with nonproductive cough.

On admission, the patient was somewhat lethargic. Bodily movements were accompanied by vertigo, with some swaying from side to side. Horizontal and vertical nystagmus, and slight lack of coordination of movements were observed. His neck was slightly rigid. Ocular examination revealed the left pupil to be slightly larger than the right. There was beginning edema of the disk, which was more in the left eye than in the right. A subcutaneous abscess, 2.5 cm. in diameter, was present on the mesial aspect of the upper third of the left leg. Spinal fluid, withdrawn under slightly increased pressure (240 mm. of water), was normally clear, the Wassermann reaction of the spinal fluid was negative, a globulin test was positive, total protein was 70 mg. in each 100 cc., lymphocytes numbered 29 and polymorphonuclear leucocytes 3 in each cubic millimeter. The gold sol curve was of the zone 1 type. Roentgenograms of the head, thorax and left tibia gave negative results. The Wassermann reaction of the blood was negative. The blood and urine were normal. The blood pressure in millimeters of mercury was 126 systolic and 78 diastolic, the pulse rate was 48 beats each minute and the temperature was 98° F.



During the patient's stay in hospital his condition was about the same as on admission. The outstanding symptoms were rigidity of the neck, nystagmus and vomiting. March 17, 1931, the subcutaneous abscess of the left leg was aspirated and 10 cc of pus was obtained. Bacteriologic examination of the pus was negative for *Mycobacterium tuberculosis* by smears and inoculation of guinea pigs. In brain broth and blood agar plates there was no growth of microorganisms. The temperature chart revealed an irregular curve, mostly within normal or subnormal range. On three occasions it reached 99° F and twice it was 99.5° F. The pulse rate ranged between 60 and 70, the respiratory rate was normal. March 27, the abscess of the leg was practically healed and since there was no change in the general progress of the disease which, in spite of repeated tests, would render the diagnosis positive, the patient returned to the care of his home physician. The diagnosis was held to be indeterminate, but there was clinical evidence of an infratentorial intracranial lesion.

At home, the patient's condition remained substantially unchanged. A slight cough developed, which became paroxysmal, dry, and never productive. The abscess of the leg returned, and drained spontaneously through multiple small sinus tracts. The skin over the abscess, except for the minute sinuses, remained intact. A second lesion, similar to the abscess of the leg, appeared over the sacrum, and likewise drained spontaneously. The temperature, pulse rate and respirations, and general mental and physical state remained about as they had been during the patient's stay at the clinic. He died unexpectedly May 14, 1931. Necropsy was performed eighteen hours after death. Vascular embalming had been done. The essential observations were as follows:

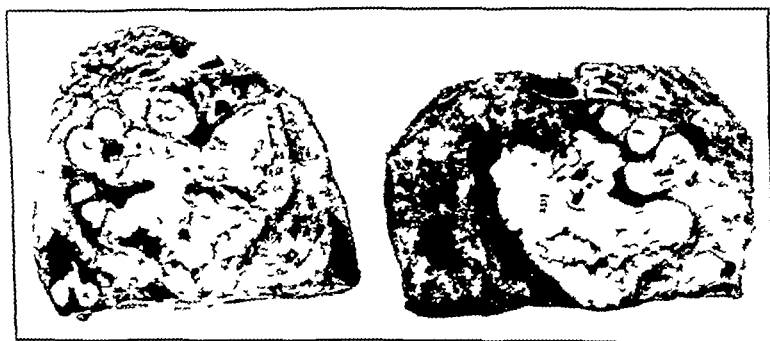


Fig 2 —Multilocular caseous mass in the lower lobe of the right lung

**Macroscopic Examination** —On the mesial aspect of the head of the left tibia there was a subcutaneous area of suppuration measuring 3 cm in diameter. It was only slightly elevated, and in its central part was punctured by multiple small sinuses from which seropurulent exudate could be expressed. The suppuration extended to the periosteum but did not involve the bone. A similar lesion was found over the sacrum.

In the upper lobe of the right lung, along the lateral margin, there was an abscess 1.5 cm in diameter, containing thick purulent material with but slight reaction surrounding it. There were other, firm hemorrhagic nodules measuring 1.5 cm in diameter, throughout the upper and lower lobes. In the right lower lobe there was a circumscribed, ovoid portion of consolidation, the pleura over the area was hyalinized and thickened. On section this mass measured 6 cm in diameter and presented the appearance of a multilocular zone of necrosis, the cavities contained caseous material. The margins of this zone were not heavily encapsulated, although condensation and consolidation of the parenchyma of the lung was evident at the periphery which was intensely hemorrhagic in its outer aspect and tended to be grayish as the zone of necrosis was approached (Fig 2). Separate

from the large mass, but nearby, there were a few firm grayish-white nodules, 0.5 cm in diameter, but without central softening. The lower lobe of the left lung exhibited indistinct zones of increased consistence, presenting a slightly nodular appearance, as of bronchopneumonia. Peribronchial lymph nodes were enlarged and on section revealed areas of apparent fibrosis and caseation. Deposits of calcium could be detected in some areas.

The ventral surface of the right lobe of the cerebellum was of firm consistence, with obliteration of the cerebellar convolutions. On section the area had the appearance of a multilocular abscess, containing zones of caseation and other areas of suppuration. There was no evidence of meningitis except locally over the lesion just described. The remainder of the brain appeared normal.

From the macroscopic appearance of the lesions, it was apparent that we were dealing with granulomatous disease. Microscopic examination of pus and caseous material from the lungs mounted in 10 per cent potassium hydroxide revealed numerous doubly contoured refractive bodies.

*Histologic Examination*—The microscopic study was made from tissues fixed in Orth's fluid. Because of previous embalming the Zenker fixation for the Mallory stains was substituted by using the Weigert mordant as described by Kernohan. Staining methods included hematoxylin and eosin, scarlach 1, Brown-Giam, carbolfuchsin-methylene blue for *Mycobacterium tuberculosis*, and Mallory's aniline blue as modified by Wade.

*Abscess left leg* The stratum corneum revealed an abnormal hyperconification with overlying crusts consisting of cellular debris, leucocytes and fibrin. In places, apparently corresponding to the small sinus tracts, the dermis and epidermis were separated by ulceration and sloughing. Adjacent to these tracts there was some extension of the ulceration, and granulation tissue arising from the dermis formed the base of the ulcer. The epithelium at the borders of the ulcer was hyperplastic. The hyperplastic reaction also involved the epithelium of some of the hair follicles. In the deeper layers of the dermis there were clusters of epithelial cells exhibiting a pseudopearl formation or presenting necrotic or abscess centers. Corresponding to the epidermal hyperplasia, the dermal papillae were of irregular morphology, revealing edema, congestion and leucocytic infiltration. The cellular collections predominated with polymorphonuclear leucocytes, among which were many basophils, but regularly present were lymphocytes, large monocytes and plasma cells. Giant cells were also conspicuous. Small abscesses or diffuse cellular reactions were the rule rather than tubercles. Lying free in the tissue spaces, or contained within giant cells, large numbers of ovoid or spherical cells were found. These presented a double refractile outer zone and a clear central protoplasm or one in which from five to six acidophilic or basophilic granules appeared. The granules were central or peripheral. Some forms contained refractive unstained bodies in their cytoplasm, usually from four to six being found. Budding could not be ascertained with certainty, although a few forms presented knob-like swellings on one side, and others appeared as pairs of cells.

*Lung* What appeared grossly as a multilocular granuloma, was confirmed. Preparations from the large multilocular area revealed the cavities to be filled with necrotic detritus, having a hyaline appearance and staining diffusely with eosin in the hematoxylin and eosin preparations. In the hyaline material, well formed cells

were rarely seen, only an occasional lymphocyte or refractive yeast-like form being observed. At the periphery of the multilocular portions basic staining cellular debris blended gradually into the living reaction zone. In the zone of tissue reaction there was granulation tissue composed of innumerable vascular channels, supported in a tissue framework of fibroblasts and endothelioid cells. Lymphocytes, monocytes, and occasional plasma cells and polymorphonuclear leucocytes were in abundance. Giant cells were also fairly numerous. Farther away from the large necrotic zone, daughter lesions were present, apparently as outgrowths from the older lesions. They were in the form of tubercles, with endothelioid and giant cells prominently displayed. Within the giant cells and lying in the interstices of the tissue there were numerous yeast-like forms.

**Peribronchial lymph nodes.** The lesions in the peribronchial lymph nodes were similar to those in the lung. Formation of tubercles with central necrosis and peripheral granulomatous reactions predominated the picture (Fig 3). The



Fig 3—Tubercle formation with central necrosis in a peribronchial lymph node (hematoxylin and eosin)

necrosis was perhaps more extensive than elsewhere, as also were the daughter tubercles. The great resemblance of the tubercles to those of tuberculosis was also a noteworthy feature. Yeast-like cells appeared free in the necrotic centers, lying in the peripheral exudate and granulation tissue and within the giant cells.

**Cerebellum.** In the cerebellum the lesions presented central necrosis in which cellular detritus was visible here and there. At the periphery of the necrotic lesions collections of polymorphonuclear leucocytes were encountered which were, in some areas, aggregated as milary abscesses. The encapsulations of both the larger necrotic areas and the milary abscesses were of granulomatous tissue, consisting of young connective tissue cells, endothelioid cells and small blood vessels, and in which lymphocytes and other leucocytes were collected. Giant cells were also prominent (Fig 4). In the necrotic areas few refractive yeast-like cells were observed, but in the milary abscesses and granulomatous tissue these fungi were very numerous, either lying free in the exudate or in the granulomatous areas of reaction, or were contained within the cytoplasm of the giant cells (Fig 5). The cerebellar tissue which surrounded the zone of inflammation was not immediately

normal but revealed necrosis and compression grading gradually to the normal, evidencing the progressive character of the lesion as it extended peripherally into the previously uninvolved tissues

**The organisms** The organisms in the cerebellar lesion were sufficiently distinctive to warrant a more detailed description. They usually appeared as spherical cells, a few, however, were oval, and with hematoxylin and eosin staining they exhibited the customary double contoured highly refractive capsule. The endo-

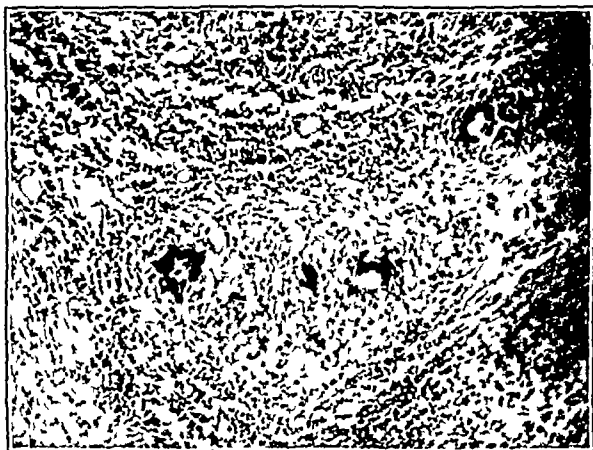


Fig 4—Confluent tubercles in the cerebellum showing giant cells and the surrounding cellular collections and vascularity (hematoxylin and eosin)

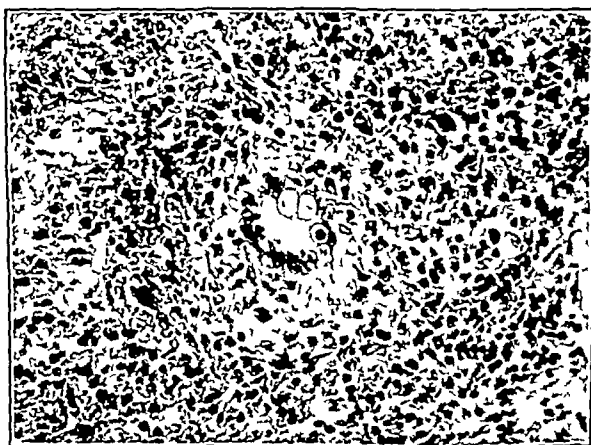


Fig 5—Organisms enclosed within a giant cell from the cerebellum (hematoxylin and eosin)

plasmic portion was revealed as either unstained, faintly basic stained and homogeneous, or granular, sometimes containing ill-defined vacuolated bodies. The size of the cells was quite variable, the predominating measurement being 10 microns with limits from 3 to 25 microns. By use of the aniline-blue staining technic, the capsule and protoplasmic portions of the cells were revealed in much greater detail. The capsule appeared as a homogeneous, basic staining zone with slightly more deeply staining outer and inner membranes. It varied in thickness proportionately with the size and age of the cells. Sometimes there appeared to be an adventitious

capsule. In most instances a clear, unstained refractive space was present immediately within the capsule, and in others this space was occupied by a condensation of finely divided basic staining cytoplasm which sometimes was dotted by acidophilic or deeply basophilic granules. In other cells the cytoplasm was similar, but was distributed uniformly and left neither outer clear zone nor central vacuolization. The cytoplasmic granules were often small and basic staining or larger and more

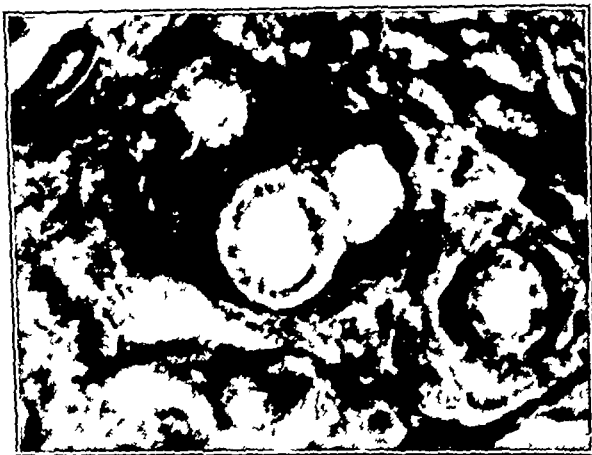


Fig 6—Organism with thick capsule and three large refractile bodies in a nearly homogeneous cytoplasm (Mallory's aniline blue)

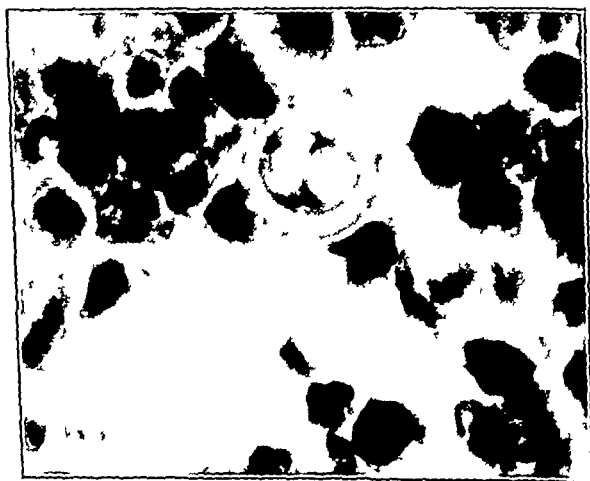


Fig 7—Two organisms with a single large central vacuole surrounded by a zone of granular spore-like bodies (Mallory's aniline blue)

clearly defined deeply basophilic or acidophilic bodies. The granules were larger and more numerous in the larger cells. The number of granules varied from one or two up to ten or more (Fig 6). In the largest cells, the center of the granule was sometimes vacuolited and refractive. In addition to the granules, refractive spherules were frequently seen in the cytoplasm, large ones as already described for the central areas and also multiple smaller ones (Fig 7). The number present was inconstant, there being frequently two, four or more, to innumerable ones. It

was impossible to stain these refractive spherules by either acid-base dyes or schi-  
lach 1, but in some there was a more deeply staining cytoplasm immediately sur-  
rounding the sphere. In some cells the granules were regularly arranged in rosette  
forms. To add further proof that these were endospores and not pseudospores it  
would be necessary to describe their further evolution. A few empty capsules could  
be found, which appeared as if the cell had discharged its contents by rupture

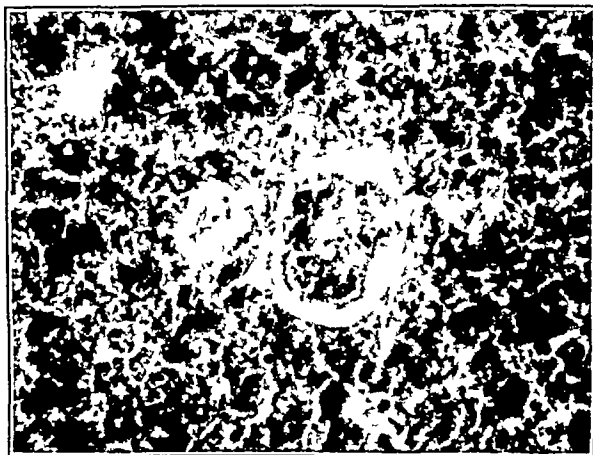


Fig. 8—A large organism filled with heavy granules, with rupture of capsule and apparent evacuation of spore-like bodies into surrounding tissue (Mallory's aniline blue)

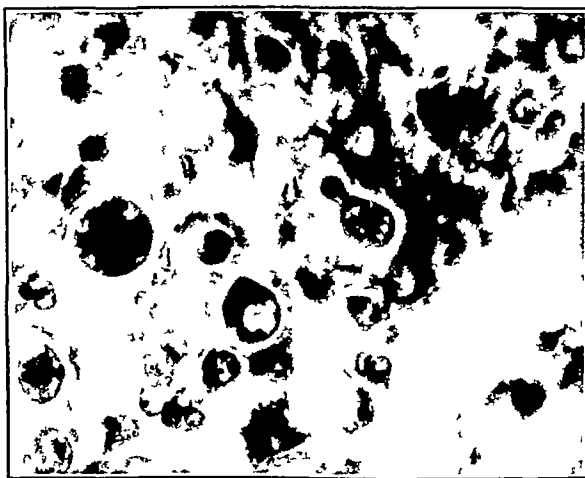


Fig. 9—Well developed budding form (Mallory's aniline blue)

The actual liberation of spores from the ruptured cell was not observed with cer-  
tainty, though in a few instances spore-like granules could be seen having the ap-  
pearance of being extruded from the ruptured cell (Fig. 8), and clusters of very  
small yeast-like cells were occasionally found.

The preparation stained with aniline-blue, besides revealing the spore-like  
forms, presented, as in the other lesions of the case, a few forms apparently in the  
various stages of budding. As revealed in the various microscopic fields, the earli-  
est evidence of budding consisted of condensation of the inner cytoplasm into a

small knob-like excrescence, occupying a position in the peripheral refractive clear space. Earlier there was no change in the heavy outer capsule. In the enlarged buds, however, the capsule was compromised, apparently to make way for this growth. Daughter cells were thus formed, appearing exactly as the parent, in miniature (Fig 9). Finally they were completely separated and surrounded by an independent capsule. The same appearance of protoplasmic granules, spore-like spherules and vacuoles could be seen in the apparent budding forms as in the nonbudding forms (Fig 10). It could be possible that these forms, rather than representing stages of budding, were preparing for rupture of the spore with liberation of the endospores, this, however, seems improbable in view of the successive stages of budding which were encountered.

Stains for *Mycobacterium tuberculosis* were negative in all of the lesions.

*Comment*—The classification of the parasite in this case was rendered more difficult because opportunity to study the cultural characteristics of the organism was lost by embalming previous to necropsy. It was necessary, therefore, for pur-

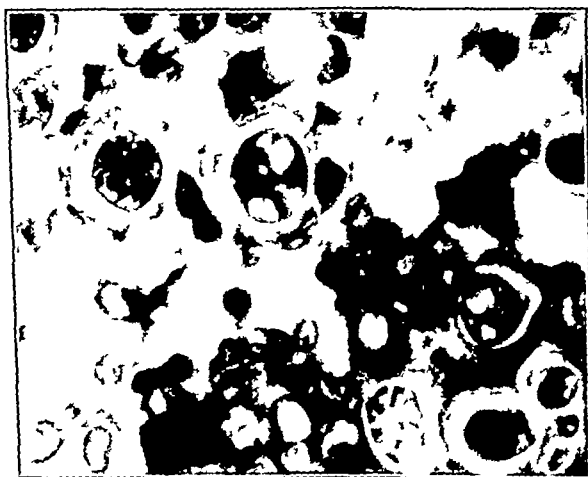


Fig 10—Organisms with vacuoles and spore-like bodies in cytoplasm. Two show early budding (Mallory's aniline blue)

poses of classification, to rely entirely on the details of the lesions and the morphologic features of the organism.

The diagnosis of torulosis was first and most readily excluded. In torulosis the organisms occur in zooglyphic masses due to a gelatinous matrix, there was no suggestion of this in our case. That the disease was not saccharomycosis of the Busse-Buschke or Curtis type could not be dismissed so easily. The cells were generally larger than have been described in the true yeast infections. The extreme rarity of the true yeast infection was also opposed to its classification as such.

The classification then rested between blastomycosis or coccidioidal granuloma. From the clinical features of the disease and from the gross and microscopic pathologic characteristics, the diagnosis of coccidioidal granuloma is strongly suggested. The short duration of symptoms, the marked resemblance of the lesions to tuberculosis, and the extensive involvement of the peribronchial lymph nodes are more nearly like coccidioidal granuloma than blastomycosis. The diagnosis of

coccidioidal granuloma receives added support from the morphology of the parasite in the tissues, especially from the occurrence of the spore-like bodies. Budding, however, is considered to be a condition which negates such a diagnosis. Nevertheless, since the greatest tendency of both the organism and the disease in our case was toward coccidioidal granuloma, we have elected that as a presumptive diagnosis.

In consideration of the occurrence of apparent endogenous sporulation and budding by the same organism it has been necessary, as indicated by our term "presumptive diagnosis" to refer to this as a case of coccidioidal granuloma with some reservation, but since we have elected the position that all organisms of this group are probably mutations of a more highly developed form such as *Endomyces*, various morphologic combinations could occur thus rendering an exact systematic classification impossible when they do not conform with so called standard concepts. These organisms, so far as our knowledge of them and homologous non-pathogens is developed, may assume various modes of growth and biologic reactions depending on environmental influences, as previously noted.

The diagnosis of coccidioidal granuloma has hitherto, to our knowledge, never been made in residents of Minnesota. More than 80 per cent of the known cases have originated in California. This adds considerable interest to this report, indicating that an organism capable of evoking a disease similar to that ordinarily endemic in California may also be found in Minnesota. That its occurrence in Minnesota and elsewhere outside of California should be anticipated is indicated from isolated reports from other localities where occasional cases of coccidioidal disease have developed in patients who had never been in California and from knowledge of the biology of the group as previously considered.

Nothing could be determined regarding the source of the infection in our case. No other cases were known in the locality from which the patient came. The primary lesion was possibly pulmonary, with lymphatic dissemination to the peribronchial lymph nodes, and hematogenous distributions to the cerebellum and cutaneous tissue. The lesion on the left leg, the other possible primary, appeared after the cerebellar disturbance became manifest. It also resembled closely an embolic lesion, in that it originated as a subcutaneous abscess without exhibiting extensive ulcerative or venous characteristics.

#### SUMMARY AND CONCLUSIONS

An unusual case of systemic infection due to yeast-like parasites has been presented. The case is of particular interest because of its organic localizations, which were cerebellar, pulmonic and subcutaneous (two small abscesses). Its clinical, etiologic, and pathologic resemblance to coccidioidal granuloma in a resident of Minnesota, who had not recently travelled elsewhere is also noteworthy. In this case, the organisms revealed dual processes of reproduction in the tissues, namely, by budding and sporulation, such as has been demonstrated by Ricketts, Stober, Montgomery, LeCount and Myers, and others. The literature on the subject of infections due to yeasts and yeast-like organisms has been partly reviewed. The review demonstrates that a close relationship exists between the various yeasts and yeast-like pathogens and the diseases which they cause, and that attempts to classify without keeping this relationship in mind tends to confuse rather than clarify the subject.



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## SULPHEMOGLOBINEMIA\*

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**S**ULPHEMOGLOBINEMIA may be defined as a condition in which the normal blood pigment hemoglobin is replaced in part by sulphhemoglobin. It is considered to be a rare condition, only 38 cases having been reported in the literature, 15 from the United States, the remainder from abroad. The authors feel however that it is much more common and that the diagnosis is frequently overlooked especially in those cases where the symptoms are mild. The two cases in this review were discovered accidentally while in consultation for a totally unrelated symptom.

The literature relating to sulphhemoglobinemia is meager. In 1902 Stokvis<sup>1</sup> reported a case of cyanosis associated with a chronic dysentery and without cardiac or pulmonary complications. On examination of the blood he found an absorption band in the red portion of the spectrum in addition to the normal bands of oxyhemoglobin. Thus he called methemoglobin but for reasons described later in this paper we believe that it was sulphhemoglobin. He dignified the condition with the title "Enterogenous Cyanosis" and assumed that absorption of toxins in this case of chronic dysentery was sufficient to produce the abnormal pigment. In the same year Talma<sup>2</sup> described two similar cases of cyanosis, one of which was accompanied by a diarrhea. In his report he described the same absorption band in the red part of the spectrum. Further he ascertained that the abnormal pigment resided in the red corpuscles and not the plasma.

Three years later van den Beigh<sup>3</sup> while studying 2 cases of enterogenous cyanosis found that one was caused by methemoglobin the other by sulphhemoglobin. This was the first recognition of the clinical entity, sulphhemoglobinemia. The distinction was made by spectroscopic examination of the blood and the theory of aniline derivative ingestion as a cause of methemoglobinemia was established by him.

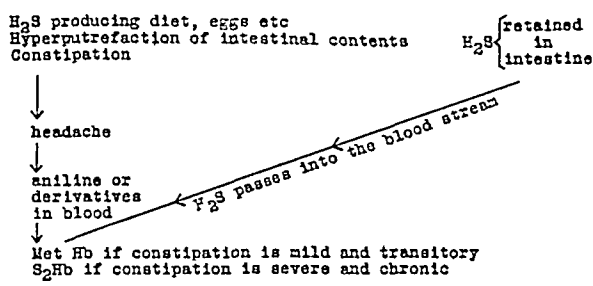
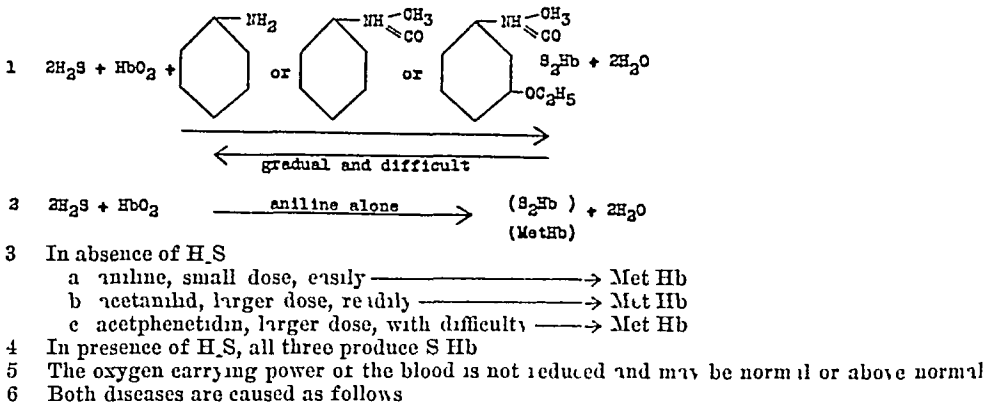
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The pathogenesis of sulphhemoglobinemia has been studied intensively for the past twenty years and several probable theories have been evolved. Lewin<sup>4</sup> before the recognition of such a condition described the absorption of  $H_2S$  after its oral, subcutaneous or intravenous administration and proved its presence in the blood and other tissues. Meyer<sup>5</sup> in a similar manner detected sulphides in the blood after rectal introduction of dihydrogen sulphide. Clarke and Huntley<sup>6</sup> in 1907 showed that the presence of powerful reducing agents such as the nitrites together with dihydrogen sulphide in the blood caused sulphhemoglobinemia. Later Wallis<sup>7</sup> studying this condition found a nitrite producing bacillus in the mouths of affected persons, the absorption of nitrites formed in this way being sufficient to cause reduction. Similar organisms were obtained from cultures of the stools. Several other workers following the same technique have been unable to substantiate his claims. In 1925, Snapper<sup>8</sup> showed by clinical and experimental means that one of the aniline derivatives, acetphenetidin, in large doses would produce sulphhemoglobinemia. Furthermore he found that methemoglobin was formed with great difficulty in those patients and animals taking aniline derivatives. The feeding of sulphur by mouth hastened the formation of sulphhemoglobin. He elaborated a theory that hemoglobin is sensitized by the aniline and can unite with sulphides absorbed from the intestine to form the new pigment.

This appears to be a fair conclusion for unless the hemoglobin were changed in some way by an exogenous agent the condition would be protean. Ten per cent of ingested sulphur is absorbed and, taking into consideration a limited intake and measured output, the difference in urinary sulphur is due to the oxidation products of dihydrogen sulphide formed in the intestine by bacterial action.<sup>9</sup> Denis and Reed<sup>10</sup> have proved further that dihydrogen sulphide introduced into the intestine is readily absorbed and causes a change in the respiratory rate in a few minutes. They detected the gas in the blood and ascribed its fate to oxidation to sulphuric acid and sulphates. Fortunately most of the dihydrogen sulphide formed in the body is located in the colon, the site of bacterial action, for these same authors found the absorption rate much greater in the small intestine.

Since the absorption of dihydrogen sulphide from the intestine in a concentration above that which the organism can oxidize and eliminate easily is necessary to cause the condition, one should expect either a large dihydrogen sulphide production or an obstruction to fecal outlet. This latter fact has been proved experimentally and clinically, a majority of the patients having been affected with severe and persistent constipation. In connection with Snapper's theory of hemoglobin sensitization to sulphur by means of aniline it is interesting to note the work of Harrop and Waterfield.<sup>11</sup> They produced sulphhemoglobinemia in splenectomized dogs by feeding sulphur without an aniline derivative. This suggests some alteration in the hemoglobin molecule which allows union with sulphur after splenectomy, and suggests a possible splenic dysfunction in those cases of sulphhemoglobinemia which have a history of no drug ingestion. Mason and Conroy<sup>12</sup> reviewing thirteen cases failed to find any evidence of coal tar product medication. To acetphenetidin, Harrop and Waterfield<sup>11</sup> add acetanilid and exposure to aniline as etiologic agents. In both our cases the habitual and excessive use of bromoseltzer was the factor. Diagrammatically the blood pigment changes are as follows,



REPORT OF CASES

CASE 1—A C, a male accountant, fifty years of age, was seen on October 20, 1931, complaining of pain in the left side of the chest. A diagnosis of pleurodynia was made and treatment gave prompt relief.

A day later a history of addiction to acetanilid was obtained. Six years ago the patient's wife died after a long illness and during that period he was obliged to do considerable of his office work at night. He had frequent frontal headaches after a week, more noticeable at night and relieved by rest of the eyes. Realizing the association of eyestrain to headaches he planned to see an oculist but on account of business pressure postponed the examination and began to take bromoseltzer for relief. At first he averaged two doses or about 5 grains of acetanilid a night and in a few weeks took as much as 10 doses a day. The headaches became generalized and it gradually became necessary to take a full dose of the drug every hour to insure comfort, the patient always carrying a medium sized bottle of bromoseltzer in his pocket for use when unable to go to a soda fountain. Six months later he had his eyes refracted and glasses changed but the headaches persisted. They were never severe enough to keep him awake but on arising he was conscious of the discomfort and would take a generous dose of bromoseltzer before breakfast. Immediately the headache subsided and then recurred in an hour necessitating more of the drug, and so on for the rest of the day.

Two or three times he had made sincere attempts to break the habit but irritability, sleeplessness, inability to concentrate on his work, an intense desire for a salty taste in his mouth and the headache would destroy his resolutions. Peculiarly enough, in the later stages the first three of the above symptoms were the ones which influenced him greatly.

The past history was entirely negative except for severe constipation since childhood, the control of which necessitated a daily enema or purge.

On physical examination the skin was found to be pale and dry, the mucous membranes of mouth cyanosed, as were also the nail beds. Aside from pyorrhea alveolaris and general hyperactive reflexes the examination was negative.

Urine was normal.

Blood examination. Red cell count, 5,200,000, hemoglobin 88 per cent by the Sahli method, white cell count 9400, morphology of corpuscles and differential count were normal. Spectro-

scopic examination of the blood revealed the typical absorption band of sulphhemoglobin and the other typical reactions outlined below

A diagnosis of sulphhemoglobinemia was made and a diet outlined. Eggs were restricted and a high fat intake prescribed. Acting on Long's<sup>14</sup> observations, inhalations of oxygen and carbon dioxide were given the first day at six hour intervals, 3 treatments in all, each lasting twelve minutes in which time the respiratory rate rose to 36 per minute. On the second day the same number of inhalations were given but the time interval required to reach a rate of 36 respirations a minute was decreased in the latter part of the day to nine minutes. The cyanosis was scarcely apparent on the third day of treatment but the spectroscopic test for sulphhemoglobin was still positive. The duration of oxygen and carbon dioxide inhalation was decreased steadily to two minutes on the fifth day, at which time the cyanosis was absent and the test for sulphhemoglobin was negative. During the period of treatment ammonium chloride was administered in 15 grain doses four times a day, and the patient abstained from bromoseltzer. To ensure catharsis a half ounce of magnesium sulphate was given by mouth. Small doses of codeine were sufficient to control the headache.

Two days after the cessation of treatment the headache disappeared and for the past two months he has had no recurrences. Frequent spectroscopic examinations of the blood have failed to show the presence of sulphhemoglobin.

CASE 2—H W G, a forty year old male confectioner was first seen in November, 1931. At that time he came in complaining of sugar in the urine, the glycosuria having been discovered during a routine insurance examination. Previously he had been in excellent health and except for frequent headaches the past history was entirely negative. The patient did not volunteer the information about his headaches and this was obtained only by questioning. For these he had used bromoseltzer and had been accustomed to take from 4 to 6 doses of the drug a day. This had gone on for several years and the patient was unable to say whether his symptoms antedated the habit. He had been chronically constipated "all his life" and took 3 alophen tablets before retiring each day. He admitted unnatural irritability for the past year, some insomnia, a tendency to become upset by trivial affairs, and complained of the changed attitude of his family and acquaintances.

Physical examination revealed a well developed and well nourished man apparently in excellent health. The skin and mucous membranes were thought to be normal in color at the first examination, but later, after the blood tests, a slight mauve lavender cyanosis was noticed on the lips. Otherwise there was no evidence of pathology.

The urine contained 0.8 per cent of sugar and a twenty four hour specimen contained 16 gm. Fasting blood sugar was found to be 140 mg/100 c.c. and a sugar tolerance test after the ingestion of 100 gm of glucose gave the following results: 131/100 and 186 mg/100 c.c. On account of the history of bromoseltzer addiction, a spectroscopic examination of laked blood was done and it revealed sulphhemoglobin.

Treatment as in Case 1 was instituted with practically identical results in the length of time of inhalations and the decreasing tolerance to CO<sub>2</sub>. The glycosuria was absent on the second day, and at the end of a week both headache and sulphhemoglobinemia were absent. The patient returned to his regular diet but the glycosuria failed to reappear. A fasting blood sugar on the tenth day was found to be 96 mg/100 c.c., and a sugar tolerance test proved to be normal a day later. Daily urinalysis and one blood sugar test have been found to be normal one month since treatment was discontinued.

#### DIAGNOSIS

Headache is the most common single symptom as reported in the literature and occurred in both our cases. Our patients described it as a dull oppressive pain, generalized, present at all hours of the day and night, and relieved by bromoseltzer for about an hour.









Constipation in both cases was severe and of long duration. Each patient volunteered the information that a purgative was required every day to insure defecation. In the reported cases this has been a consistent symptom.

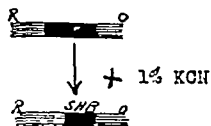
Cyanosis in the absence of other symptoms or signs of heart or lung disease is a characteristic finding in these cases. The cyanosis is mauve-lavender as described by Harrop and Waterfield<sup>11</sup> and is due to the abnormal pigment in the blood.

Nervous symptoms were marked in both cases. Patient I was definitely of a neurotic type and recognized by his neighbors as "peculiar." For several years he had made himself a burden to relatives and neighbors because of his many manifestations of emotional and nervous instability.

The only single method of making the diagnosis is by spectroscopic examination of the blood, either by means of a hand spectroscope through the lobe of an ear or directly viewing the diluted and laked blood in a tube. In this way all parts of the spectrum except the red and orange are cut out and the band of sulphhemoglobin can be seen slightly nearer the orange than the band of methemoglobin. The disappearance of the band after the addition of ammonium sulphide T. S. to the laked blood indicates that the abnormal pigment is methemoglobin. Its persistence confirms the diagnosis of sulphhemoglobinemia. The diagnosis of sulphhemoglobinemia may be tabulated as follows:

$S_2$ Hb aemia	Met Hb aemia
a mauve-lavender cyanosis, due to mauve-lavender blood	a blue chocolate brown cyanosis, due to the same color of the blood
b weeks required for cyanosis to fade away gradually upon discontinuance of the drug	b forty-eight hours required for the cyanosis to disappear entirely upon discontinuance of the drug

$S_2$ -Hb-aemia	Met-Hb-aemia
c spectroscopic tests	c. spectroscopic tests
1 by pocket spectroscope see light thru ear 	1 same way 
2 draw a little blood from ear and dilute with water in a small tube. Examine the spectrum 	2 same way 
3 if dilute $(NH_4)_2S$ , 1% KON are added to the above diluted blood 	3 same way 
4 if CO is bubbled thru the original diluted blood 	4 same way 
5 measure the wave-length 620-607	5 measure the wave-length 618-630
6 When both $S_2$ -Hb and Met-Hb are present the two absorption bands overlap. Divide the diluted blood into two tubes and add to one of them a drop of 1% KON. It is the band of Met-Hb that disappears	



Jameson<sup>13</sup> described a spontaneous and rapid cure of the condition, without treatment the case being of unknown etiology. Van den Bergh's case which we may consider to be sulphhemoglobinemia was cured by operative treatment of a rectal stricture. Wallis used an autogenous nitroso-bacillus vaccine successfully and Garrod<sup>14</sup> reported satisfactory results in the treatment of a combined methemoglobinemia and sulphhemoglobinemia by the same type of vaccine and withdrawal of drugs. In the latter case the cyanosis disappeared after a month of treatment. Long and Spriggs<sup>15</sup> used oxygen inhalations during a fainting attack in one of these cases and noticed the improvement in the cyanosis. All authors advise purgatives, withdrawal of any offending drugs, a high carbohydrate diet, and a low sulphur intake.

To these the author wishes to add the use of oxygen and carbon dioxide inhalations supplemented by a high fat diet and the administration of ammonium chloride by mouth, 15 grains four times a day. The ordinary cylinders used by anesthetists containing 5 per cent carbon dioxide in pure oxygen and a mask of the type used in nitrous oxide work is the only apparatus required. The inhalations were given two or more times a day and continued until the respiratory rate reached thirty-six and the respirations were definitely hyperpneic, or until signs of CO<sub>2</sub> poisoning became apparent. As the cyanosis diminished, the tolerance to carbon dioxide decreased and on the last day of treatment the inhalations could be given for only two minutes.

The prophylactic treatment is important and embraces a policy of educating the public in the dangers of these drugs and a policy of conservatism in prescribing acetphenetidin and acetanilid.

#### CONCLUSIONS

- 1 A brief review of the literature and the theories of the pathogenesis of sulphhemoglobinemia is presented.
- 2 Two cases with typical symptoms and signs of the condition are described.
- 3 A new method of treatment, inexpensive, and simple of application, which shortens the curative period from weeks to days is described.

The author wishes to express his appreciation to Mr John D. A. Gatsos, A. B., for his assistance in the preparation of this article.

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## TARTRATE METABOLISM\*

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### I GRAPE TARTRATES AND THE ACID-BASE BALANCE

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IT is now quite generally understood that maintenance of the acid-base balance is necessary for continued good health and that restoration of a disturbed balance is a valuable therapeutic measure in dealing with certain types of disease. A shift toward the acid side is sometimes desirable, an instance being the pre-medication which has been found useful in making urotropin active as a urinary antiseptic, but we much more frequently hear of alkalinizing the body, and in bringing about such a change diet is becoming increasingly useful. Many fruits and vegetables are rich in chemical compounds of the desired type, the most efficient being sodium or potassium salts of a few of the organic acids.

Biochemists have found that the salts that are effective alkalinizers undergo in nearly all cases as the first step in metabolism one of three processes: their organic acid radicals may be oxidized or otherwise decomposed, may unite with basic ions other than sodium or potassium to form insoluble or nonionized compounds, or may be joined with ammonia to form ammonium salts ready for excretion. In any of these events, sodium or potassium ions become available as active alkalinizing agents.

The alkalinizing power of citrates, readily administered in the form of citrus fruits or their juices, is well known. The case is not so clear for tartrates, which compose almost the entire quantity of the salts of organic acids in grapes, and which are not elsewhere found to any great extent.

The effects of tartrates, both upon lower animals and upon the human organism, have been extensively studied, but the results obtained are as yet inconclusive, especially with respect to their influence upon the acid-base balance. As long ago as 1914, Post,<sup>1</sup> having considered the reports of several men who had been testing the action of tartrates upon animals, and having had a question raised in his mind by comparing their adverse testimony with the claims of Fischer<sup>2</sup> that tartrates are beneficial in cases of edema and nephritis, gave Rochelle salts to a series of his patients, the quantities varying from one half to three times the U S P dose. His findings were: "(1) They give no evidence indicating that potassium and sodium tartrate in ordinary doses given by mouth in the human subject will cause albuminuria or cylinduria. (2) There is no evidence to show that tartrates ag-

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gravate an existing nephritis (3) The acidity of the urine, indicated by the hydrogen ion concentration, was as a rule less after the administration of tartarates "

The work and observations of Post should have gone a long way toward settling the tartarate question, but his conclusions have not been given the weight which we feel they deserve. In 1924, Rose<sup>3</sup> reviewed much of the literature on this subject and reported some of his own work. He noted that certain investigators had recovered from the urine quantities ranging from 8 per cent to 45 per cent of the tartarates administered to animals, that they had found albumin and casts in the urine of such animals, and that autopsy of the animals and microscopic examination of their kidneys had given evidence of injury to the tubules, especially in the convoluted portions. His own observations were confirmatory, his proof of kidney damage being that the composition of the blood of animals to which tartrates had been given showed the typical changes which are generally recognized as indicating decreased kidney function. He concluded "Apparently tartrates are oxidized in the animal organism with great difficulty "

Simpson<sup>4</sup> gave tartrates to dogs, rabbits and cats, reporting the dosage calculated as tartaric acid. To dogs he gave by stomach tube from 0.35 gm. to 0.59 gm. per kg. of body weight and recovered from 17 per cent to 30 per cent from the urine. He gave subcutaneously from 0.14 gm. to 0.59 gm. per kg. and recovered from 47 per cent to 71 per cent. From rabbits he recovered a much smaller percentage of the dose introduced into the stomach, but he demonstrated that a rabbit's intestinal contents are capable of decomposing tartrates, if they are incubated together for twenty-four hours at body temperature. Cats were given from 0.54 gm. to 2.78 gm. per kg., and the amount recovered was from 44 per cent to 78 per cent. Animals showing a purgative effect from the tartrates excreted a smaller percentage of the dose through the kidneys, as might be expected. One of Simpson's conclusions was similar to that of Rose, but even stronger. He said "It is, therefore, unlikely that tartaric acid is oxidized in the tissues "

Within the past few years many conflicting opinions about tartrates have been published. In 1926 a standard work<sup>5</sup> contained the following words "The tartrates differ from the salts of most of the other vegetable acids in that they are not oxidized in the body but are eliminated unchanged through the kidney. It follows that they do not act as systemic alkalinizers as do the citrates and acetates. The tartrates are also less readily absorbed from the intestines than the citrates, "

In 1927, Webster<sup>6</sup> published a small volume in which he gave a comprehensive review, with numerous abstracts, of the tartarate literature of our own and several other countries. Referring to an article by Curtis in the "Reference Handbook of Medicine," 1917 edition, and speaking of Rochelle salts, he said "In non-purgative doses, especially if given well diluted, it is absorbed, changes to carbonate, then exerts, as efficiently as other salts, the specific action of alkaline potassium compounds." He also referred to the "British Pharmaceutical Codex," 1923 edition, page 60, and stated "Tartaric acid is wholly or partly neutralized in the intestine, and a small portion is absorbed, The portion absorbed is for the most part oxidized and excreted in the urine as a carbonate, which renders the urine less acid. " These statements quoted by Webster agree in

general with Post's ideas, but they manifestly contradict the foregoing quotation from the "Dispensatory of the United States of America," with respect to the tartarates as alkalinizing agents

The authors of three recent books that are now widely used as texts or references in pharmacology classes do not bring us much nearer to a solution of the tartaric problem. Solis-Cohen and Githens<sup>7</sup> say that both potassium bitartrate (the chief tartaric in grapes) and Rochelle salts are "alkalizers." McGuigan<sup>8</sup> says "Tartarates are to some extent oxidized to carbon dioxide." According to Sollmann<sup>9</sup> "The tartarates are not readily burned in the body, and therefore do not act as alkalis and diuretics as powerfully as do most other organic anions."

We should add a statement made by MacCallum<sup>10</sup> in discussing the edema believed to be due to certain poisons, for he mentions grape juice, which directly concerns our present subject. "Such substances as morphine, the juice of grapes and of various fruits, etc., are always spoken of in the textbooks as capable of producing such edema." We have not found grape juice so generally mentioned with disfavor as is here suggested, but MacCallum's statement at least serves to show its reputation among some men whose names and work are in high repute.

More recently, while our experiments were in progress, Pickens and Hetler<sup>11</sup> published their opinion that "Grape juice certainly does not function materially in decreasing the acidity of the urine." This conclusion was reached from tests made upon three normal women. It was not found that adding a pint or a quart of Welch's grape juice to a "basal" diet made any appreciable difference in the  $P_H$  value of the urine. Judging from their recorded data, the average "basal"  $P_H$  of their three subjects was about 6.

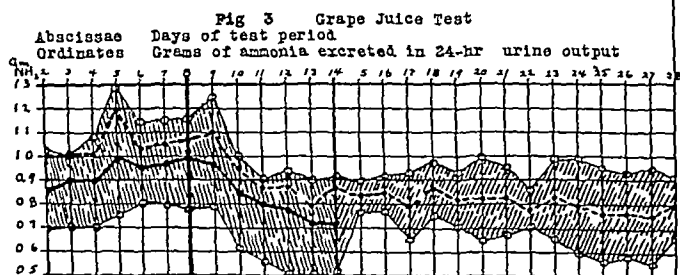
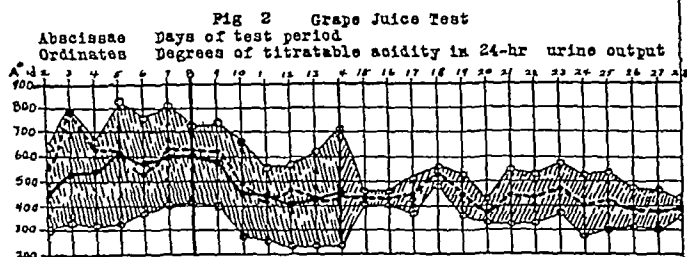
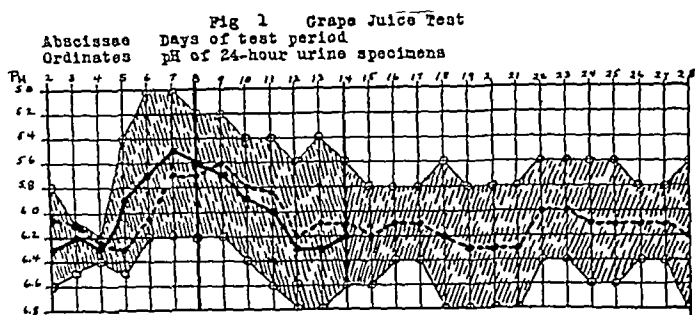
Recent contacts with dietitians and with physicians who are giving considerable attention to dietary problems have left us with the impression that not a few have the idea that tartaric containing foods, which include grapes and grape products, are of doubtful value. There seems to be quite a widespread fear that these foods may on the one hand be harmful to the kidneys and on the other be of little or no use as alkalinizing agents. We do not claim to know all the reasons for these adverse opinions about tartarates but we suspect that most of them are based upon the results of animal experimentation. In this connection we call attention to some pertinent observations of Post<sup>12</sup> (1) Doses given to animals were larger in proportion to body weight than any ordinary dose would be for a human being. (2) Toxicity was greater when the tartarates were administered

TABLE I

TEST NO	SUBJECT	WEIGHT OF GRAPES EATEN PER 24 HR PERIOD	WEIGHT OF TARTRATES IN THESE GRAPES	VOLUME OF URINE PASSED DURING 24-HR PERIOD	$P_H$ OF THE URINE FOR 24 HR PERIOD	AVERAGE $P_H$ OF URINE FOR FIVE DAYS ON NORMAL DIET
I	S	3000 G	18.9 G	1000 cc	7.0	6.4
II	S	2600 G	18.3 G	1340 cc	7.0	6.4
II	P	1850 G	13.1 G	1100 cc	6.6	6.1

subcutaneously than when they were introduced into the stomach (3) Tartarates were more toxic to fasting animals than to those that were well fed, and the type of food affected the toxicity

We do not deny the value of experiments made upon the lower animals, but we wish to emphasize the seemingly often forgotten truth that conclusions reached from such investigations are not always 100 per cent applicable to human beings



Figs 1 2 and 3—A graphic record of the results of the test with grape juice on ten human subjects. In each figure the upper limit of the shaded area represents the most strongly acid condition found in any of the ten subjects the lower limit the least acid the solid curve the average of all ten subjects and the broken curve the average of the two subjects whose test period was continued two weeks longer than the others. During the first seven days the subjects took an acid-forming control diet. The eighth day marked by the heaviest vertical line shows the beginning of the effect produced by adding grape juice to the diet. In Fig 2 one degree of acidity represents the equivalent of 1 cc of N/10 acid

Furthermore, different people vary so much in their reaction to any given set of conditions that we are constantly in danger of being too dogmatic in the generalizations that we draw from tests made upon but a few human subjects

The work described below, a preliminary report<sup>13</sup> of which has already appeared, while not as extensive as we wish it might be, was more so than any of a similar sort that we have yet seen reported in the literature. We hope it will bring all who are interested a step nearer understanding the effects of tartarates on the human body. We have used a total of twenty subjects, some more than once

TABLE II

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	PH OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
B	2	1200 cc	6.6	300°	0.75 gm
B	3	1260	6.5	328	0.70
B	4	1310	6.4	314	0.71
B	5	1400	6.4	322	0.74
B	6	1330	6.0	372	0.81
B	7	1220	5.8	403	0.79
B	8	830	5.8	415	0.77
B	9	1280	5.8	397	0.79
B	10	810	6.4	275	0.62
B	11	1270	6.6	267	0.56
B	12	1050	6.6	231	0.50
B	13	1000	6.6	230	0.50
B	14	1000	6.6	240	0.51
D	2	1340	6.4	456	1.01
D	3	1450	6.2	594	0.98
D	4	1330	6.3	492	0.93
D	5	1120	6.1	616	1.10
D	6	1340	6.2	562	0.94
D	7	1140	6.2	570	1.07
D	8	870	5.6	626	1.13
D	9	1830	5.8	549	1.06
D	10	1380	6.2	455	0.87
D	11	1700	6.2	408	0.80
D	12	1100	6.4	352	0.77
D	13	1340	6.4	362	0.71
D	14	1050	6.4	294	0.57
J	2	700	6.4	462	0.70
J	3	650	6.1	520	0.75
J	4	690	6.2	669	0.94
J	5	1200	6.3	624	0.97
J	6	890	5.6	703	0.97
J	7	960	5.4	672	1.01
J	8	840	5.4	730	0.99
J	9	640	5.4	704	0.97
J	10	600	5.4	660	0.84
J	11	830	5.6	549	0.85
J	12	1470	6.4	353	0.80
J	13	1330	6.2	399	0.77
J	14	1760	6.4	458	0.79
L	5	700	5.4	420	
L	6	570	5.2	486	
L	7	700	5.4	476	
L	10	740	6.0	320	
L	12	940	6.6	234	
L	13	850	6.7	221	
M	2	1030	5.8	639	1.03
M	3	1300	6.1	793	1.01
M	4	1550	6.2	635	1.08
M	5	1640	6.1	771	1.29
M	6	1680	5.8	604	1.14
M	7	1730	5.4	744	1.15
M	8	900	5.2	702	1.16
M	9	1830	5.4	741	1.24
M	10	1790	5.2	537	1.00
M	11	1840	5.4	405	0.86
M	12	1680	5.6	504	0.94

TABLE II (CONTINUED)

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P <sub>H</sub> OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
M	13	1500	5.4	510	0.89
M	14	2150	5.6	516	0.92
M	15	2060	5.8	453	0.89
M	16	2150	5.8	451	0.92
M	17	1650	5.8	511	0.93
M	18	2340	5.6	562	0.96
M	19	2110	5.8	527	0.93
M	20	2210	5.8	420	0.98
M	21	1930	5.8	559	0.95
M	22	1660	5.6	532	0.85
M	23	2230	5.6	580	0.98
M	24	2240	5.6	527	0.98
M	25	2350	5.6	521	0.95
M	26	2270	5.8	478	0.93
M	27	2500	5.8	475	0.94
M	28	2050	5.6	431	0.91
P	5	1550	5.4	837	
P	6	1800	5.4	684	
P	7	1500	5.4	620	
P	12	2050	6.6	565	
P	13	1150	6.0	628	
P	14	1620	6.2	713	
Q	5	1200	5.4	744	0.94
Q	6	900	5.0	764	0.98
Q	7	1000	5.0	812	0.94
Q	8	1030	5.4	576	0.87
Q	9	1700	6.2	536	0.85
Q	10	1500	6.4	468	0.81
Q	11	1750	6.4	547	0.83
Q	12	1800	6.4	468	0.84
Q	13	1800	6.4	540	0.80
R	2	1240	6.3	515	0.97
R	4	950	6.3	618	0.94
R	5	1770	6.5	561	1.10
R	6	930	6.3	465	0.94
R	7	1360	6.0	530	0.95
R	8	990	6.2	554	0.99
R	9	1180	5.8	496	0.95
R	10	1110	6.0	455	0.89
R	11	1060	6.3	445	0.90
R	12	1450	6.8	435	0.81
R	13	810	6.8	348	0.69
R	14	1120	6.6	381	0.82
R	15	1960	6.6	412	0.77
R	16	940	6.4	404	0.78
R	17	620	6.4	378	0.65
R	18	870	6.8	496	0.77
R	19	1395	6.8	363	0.71
R	20	890	6.8	329	0.65
R	21	1060	6.8	329	0.68
R	22	1040	6.4	339	0.71
R	23	1250	6.4	388	0.66
R	24	910	6.6	273	0.60
R	25	900	6.6	297	0.56
R	26	880	6.4	308	0.58
R	27	820	6.4	301	0.55
R	28	630	6.8	360	0.66

TABLE II (CONTINUED)

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P <sub>H</sub> OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
S	5	1460	5.4	681	
S	6	1350	5.4	630	
S	7	960	4.8	672	
S	12	1400	6.6	427	
S	13	1140	6.6	350	
S	14	1160	6.0	614	
T	2	930	6.2	344	0.69
T	3	940	6.2	413	0.96
T	4	930	6.3	321	0.74
T	5	810	6.2	510	0.78
T	6	640	6.0	448	0.87
T	7	1070	5.6	482	0.89
T	8	870	5.4	618	1.04
T	9	950	5.2	636	0.92
T	10	880	5.6	493	0.83
T	11	1040	5.8	478	0.78
T	12	1110	5.6	433	0.77
T	13	960	5.8	374	0.70
T	14	1130	5.6	416	0.71

They were all in good health and ranged in age from the early twenties to the early forties.

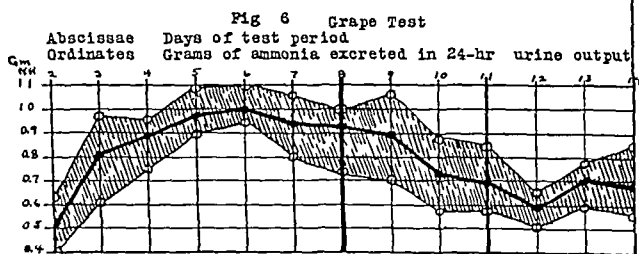
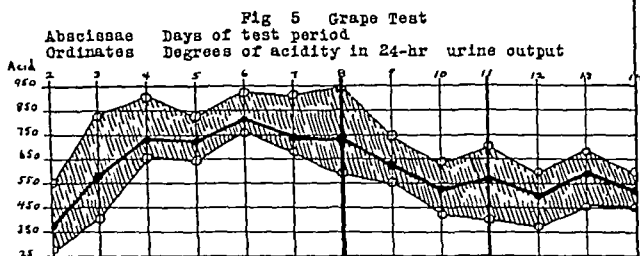
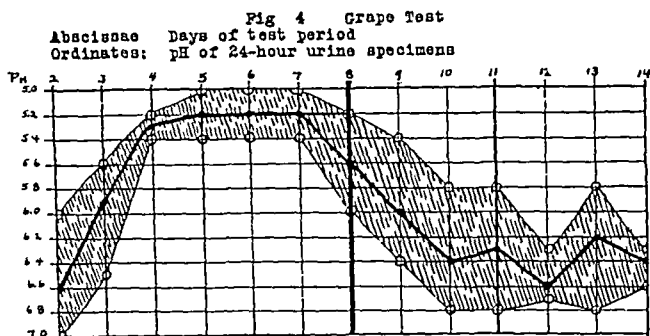
In our first two tests the urinary P<sub>H</sub> of subjects P and S was determined for five consecutive days while they were on their normal lacto-ovo-vegetarian diet. On the sixth and seventh days they ate nothing but grapes. Twenty-four-hour urine specimens were collected the seventh day. Tests were made as indicated in Table I, tartarates being calculated as potassium acid tartarate.

In the third test, subject S ate 450 gm. of Thompson seedless raisins daily for seven days, together with 50 gm. of nut margarine, three eggs, and enough bread and other cereal products to make a total diet of approximately 3000 calories a day. Water was the only liquid taken. The degree of urinary acidity that might be expected from such a diet, if raisins were left out, can be judged from the effect of our control diet for the fourth test, which was quite similar to the third test diet minus the raisins. This control diet is described in the second paragraph below. It gave subject S a urinary P<sub>H</sub> of 5.2. The quantities of urine passed for the last three days of the raisin diet period were 1200 cc., 1280 cc. and 1170 cc., respectively. The urinary P<sub>H</sub> on each of these days was 6.6.

These first three tests were made chiefly with the idea of getting data that would help us decide whether or not we should study our subject more extensively. The uniformity with which the results pointed to the alkalinizing nature of grape tartarates led us to go on. We made no more tests with raisins, however, for Blatherwick's<sup>14</sup> report about their power to reduce urinary acidity seems to be quite generally accepted and one of the lay publications<sup>15</sup> recently referred to some work done in the Fruit Products Laboratory of the University of California, showing the value of raisins in overcoming acidosis.

Subjects L, P, Q, and S were used in the fourth test. Each subject at first took a control diet for one week, composed of three or four eggs a day, 30 to 50 gm.

of butter or nut margarine, 250 to 400 gm of bread or its equivalent in other cereal foods, as much milk as desired, and occasionally small quantities of walnuts, cheese, canned green corn, or some other acid-forming or but slightly alkalinizing vegetable. During the second week the same general dietary program was continued, except for the elimination, or the reduction in quantity, of milk, which is approximately neutral with respect to the formation of acid or base in the body,



Figs 4 5 and 6—A graphic record of the results of the test with grapes on six human subjects. In each figure the upper limit of the shaded area represents the most strongly acid condition found in any of the six subjects the lower limit the least acid and the solid curve the average of all six subjects. During the first seven days the subjects took an acid-forming control diet. The eighth day marked by the heaviest vertical line shows the beginning of the effect produced by adding fresh grapes to the diet. Beginning with the eleventh day marked by the medium heavy vertical line the grapes chosen had a somewhat lower tartrate content than those previously eaten. In Fig 5 one degree of acidity represents the equivalent of 1 c c of N/10 acid.

and each subject took one liter of grape juice a day. We used a blend of the juices of Concord and Worden grapes, with a tartrate content of 0.93 per cent.

While Test IV was in progress it occurred to us that a test of urinary  $P_H$  alone would not be so conclusive as a combined check of  $P_H$ , titratable acidity and ammonia output, since all three have to do with the acid base balance. We were able to obtain data on  $P_H$  and titratable acidity from all four subjects and on ammonia output also from subject Q.

TABLE III

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	PH OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
C	2	1600 cc	6.9	336°	0.54 gm
C	3	1750	6.5	413	0.80
C	4	1020	5.2	765	0.95
C	5	1320	5.4	686	0.92
C	6	920	5.4	773	0.94
C	7	1500	5.2	915	0.99
C	8	1540	5.2	678	0.99
C	9	1320	6.0	726	0.89
C	10	1640	6.8	426	0.70
C	11	1540	6.8	400	0.63
C	12	1430	6.7	372	0.56
C	13	1360	6.4	558	0.72
C	14	1660	6.6	515	0.65
E	2	930	6.7	279	0.40
E	3	760	5.6	418	0.62
E	4	950	5.2	665	0.90
E	5	840	5.0	697	0.97
E	6	1160	5.2	801	0.94
E	7	920	5.2	672	0.88
E	8	1080	5.8	583	0.73
E	9	1100	6.2	572	0.70
E	10	1120	6.8	470	0.58
E	11	1080	6.6	454	0.58
E	12	825	6.7	422	0.51
E	13	1460	6.8	453	0.59
E	14	1460	6.6	453	0.60
F	2	650	6.3	312	0.45
F	3	1470	5.8	544	0.84
F	4	1100	5.4	660	0.85
F	5	800	5.2	640	0.89
F	6	1200	5.0	800	0.98
F	7	1380	5.0	717	1.01
F	8	900	5.6	748	0.99
F	9	1760	5.8	634	1.01
F	10	1540	6.0	547	0.88
F	11	1120	6.0	526	0.63
F	12	970	6.3	602	0.63
F	13	980	6.4	672	0.73
F	14	1600	6.3	549	0.73
G	2	1620	6.5	444	0.60
G	3	1100	5.6	825	0.97
G	4	1300	5.4	910	0.94
G	5	1100	5.4	825	1.09
G	6	1170	5.2	936	1.03
G	7	1120	5.4	672	0.89
G	8	1180	6.0	732	0.91
G	9	1600	6.4	640	0.95
G	10	900	6.2	567	0.67
G	11	1160	6.2	704	0.75
G	12	820	6.3	549	0.60
G	13	1160	5.8	678	0.71
G	14	1620	6.5	535	0.68
H	2	830	6.0	556	0.58
H	3	750	5.6	645	0.68
H	4	680	5.2	680	0.75
H	5	1000	5.0	837	0.98



TABLE III (CONTINUED)

SUBJECT	DAY OF TEST	VOLUME OF URINE PASSED	P <sub>H</sub> OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
H	6	820	5.0	800	0.97
H	7	780	5.2	749	0.80
H	8	670	5.2	943	0.99
H	9	700	5.4	560	0.70
H	10	780	5.8	640	0.65
H	11	780	5.8	632	0.72
H	12	1020	6.6	587	0.58
H	13	900	5.8	603	0.67
H	14	720	6.3	497	0.56
K	2	1300	7.0	312	0.64
K	3	1200	6.2	612	0.92
K	4	620	5.2	713	0.94
K	5	950	5.4	712	0.97
K	6	820	5.4	763	1.11
K	7	1460	5.4	730	1.05
K	8	1730	5.6	717	0.98
K	9	900	6.0	747	1.07
K	10	1000	6.8	530	0.82
K	11	960	6.6	672	0.84
K	12	1000	6.6	460	0.65
K	13	1120	6.2	547	0.78
K	14	1560	6.3	593	0.85

In completing Test IV we used six more subjects B, D, J, M, R, and T. Their preliminary diet was similar to that already described, though not so rigidly controlled, and their urine was given the triple test. M and R were asked to extend their test period until they had taken grape juice for three weeks, so that we might get a better idea of the permanence of the changes that occurred. Data obtained from the ten subjects are given in Table II, and the significant results are graphically illustrated in Figs. 1, 2, and 3.

Subjects C, E, F, G, H, and K were used in a test similar to Test IV in every respect except that 1 kg. of fresh grapes replaced each liter of grape juice. Several different varieties of grapes were used, a comparatively watery variety, low in tartrate content, forming the bulk of the grape ration the last four days of the test period. Our purpose was to see if a continuance of the grape diet, but a decrease in the tartrates ingested, would be marked by any significant change in the data obtained by our triple check on the urine passed by the six subjects. The data for this, Test V, are given in Table III, and the results are graphically shown in Figs. 4, 5, and 6.

In Test VI subjects I, N, and S took a preliminary diet similar to that used in Tests IV and V, taking also Rochelle salts (COOK-CHOH-CHOH-COONa) with every meal during the second week. The results are given in Table IV. Table V gives the data from Test VII, made on subjects A and O. Test VII was parallel to Test VI, except that potassium bitartrate (COOK-CHOH-CHOH-COOH) was used instead of Rochelle salts.

A study of Tables I to V and Figs. 1 to 6 shows that tartrates, whether given as normal or acid salts or in the form of grapes or grape products, proved to be alkalinizing in every case, though different subjects were affected to different de-

TABLE IV

SUBJECT	DAY OF TEST	DAY'S DOSE OF TARTRATES	VOLUME OF URINE PASSED	PH OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
I	2	0 gm	940 cc	6.2	583°	0.87 gm
I	3	0	720	5.6	590	0.86
I	4	0	1300	6.2	715	1.21
I	5	0	880	5.6	678	1.12
I	6	0	1300	5.8	637	1.10
I	7	0	1070	5.2	663	1.12
I	8	12	990	5.2	614	1.04
I	9	15	1240	6.2	496	0.95
I	10	15	930	6.8	307	0.60
I	11	18	1250	6.6	375	0.63
I	12	18	1170	6.8	304	0.79
I	13	20	1200	7.0	192	0.65
I	14	20	1170	6.8	281	0.54
N	2	0	1110	6.2	522	1.01
N	3	0	950	6.0	665	1.08
N	4	0	1060	5.6	668	1.21
N	5	0	875	5.8	560	0.99
N	6	0	960	5.4	595	1.30
N	7	0	1000	5.2	555	1.18
N	8	12	1510	5.2	529	1.18
N	9	15	1170	6.4	410	0.95
N	10	15	1040	6.8	270	0.65
N	11	18	1020	7.2	173	0.57
N	12	18	1020	6.6	306	0.63
N	13	20	950	7.0	262	0.53
N	14	20	1010	6.8	212	0.60
S	4	0	1620	5.2	729	0.74
S	5	0	1530	5.4	658	0.78
S	6	0	1460	5.4	637	0.75
S	7	0	1250	5.6	550	0.70
S	8	12	1850	6.4	516	0.56
S	9	12	1600	6.6	514	0.44
S	10	12	1700	6.6	510	0.49
S	11	12	1470	6.4	547	0.58

grees Rochelle salts showed the greatest alkalinizing power, and potassium bitartrate the least, but when one thinks of the chemical formulas of these two salts this difference is not hard to understand, the molecule of potassium bitartrate containing but one alkali metal atom, while the molecule of Rochelle salts contains two. With respect to the negative results obtained by Pickens and Hetler with grape juice, we make no comment except to say that we agree with a statement in the *Journal of the American Medical Association*,<sup>16</sup> which, referring to their work, said "One puzzling aspect of the behavior of the unfermented, sweetened grape juice used in these studies is the fact that raisins are known to decrease the acidity of the urine."

Chemical and microscopic tests on all urine specimens failed to show the presence of either tartrates, albumin, or casts in any case. None of the subjects complained of any ill-feeling. Grapes and grape juice caused a mild catharsis at first in a few cases, but this disappeared within two or three days. Neither Rochelle salts nor potassium bitartrate produced catharsis. Data in the tables show no noticeable diuresis as a result of taking tartrates along with a normal

TABLE V

SUBJECT	DAY OF TEST	DAY'S DOSE OF TARTRATES	VOLUME OF URINE PASSED	PH OF URINE	TITRATABLE ACIDITY	AMMONIA OUTPUT
A	2	0 gm	1050 cc	5.4	493°	0.65 gm
A	3	0	720	4.8	562	0.62
A	4	0	1000	5.0	750	0.73
A	5	0	835	4.8	726	0.78
A	6	0	670	5.2	670	0.75
A	7	0	850	4.8	655	0.88
A	8	3	890	5.0	632	0.98
A	9	6	860	5.0	636	0.91
A	10	6	740	5.2	540	0.75
A	11	9	960	5.2	643	0.78
A	12	9	750	5.4	373	0.62
A	13	12	840	5.4	398	0.66
A	14	12	750	5.6	330	0.50
O	2	0	1430	5.8	611	1.01
O	3	0	910	6.2	692	0.99
O	4	0	880	5.4	581	0.81
O	5	0	845	5.4	668	1.00
O	6	0	930	5.6	632	1.12
O	7	0	890	5.4	534	1.01
O	8	3	1550	5.4	620	1.08
O	9	6	1020	5.2	663	1.16
O	10	6	1120	5.8	560	1.03
O	11	9	1410	5.4	522	0.99
O	12	9	940	5.4	536	0.87
O	13	12	1550	5.4	527	0.84
O	14	12	1570	5.8	465	0.85

quantity of food, expect perhaps a mild degree in the case of subjects M and O. We conclude from our work, therefore, that grapes, raisins, and grape juice should be considered as harmless and effective additions to the list of foods that may be used in making up an alkalinizing diet.

#### SUMMARY

1 A series of seven tests was made on a total of twenty healthy human subjects, to determine the effect of adding grapes, raisins, grape juice, Rochelle salts, or potassium bitartrate to an otherwise acid-forming diet.

2 All these substances were definitely alkalinizing, though potassium bitartrate was less so than the others.

3 No proof of kidney damage or other injury could be found in any case.

4 Our work, therefore, indicates that grapes, raisins, and grape juice are harmless and effective alkalinizing foods.

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## TARTRATE METABOLISM\*

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### II THE MODE OF ACTION OF INGESTED TARTRATES

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IN CONNECTION with work reported in the first part of this paper<sup>1</sup> under the subtitle "Grape Tartrates and the Acid-Base Balance" there were developments which led us to study further into the way ingested tartrates are handled in the human body. We found that grapes, raisins, grape juice, and the alkali salts of tartaric acid decreased the acidity of the urine of all the subjects used in our experiments. This finding, though it differed from that of Pickens and Hetler<sup>2</sup> about grape juice, was in harmony with the results obtained by Post<sup>3</sup> with Rochelle salts and with what was long ago found to be true concerning raisins by Blatherwick<sup>4</sup> and recently by workers in the Fruit Products Laboratory of the University of California.<sup>5</sup> But, because of the uncertainty regarding the method by which tartrates produce their observed effect, an uncertainty largely due to the contradictory nature of statements found in periodicals and textbooks, we have given particular attention to three questions: (1) Are tartrates absorbed to an appreciable extent from the human intestine? (2) If absorbed, are they oxidized in the tissues? (3) If not absorbed and oxidized, how can they be alkalinizing?

If tartrates were found in the urine after ingestion by human subjects, it would be evidence of their absorption through the wall of the gastrointestinal tract and into the blood as preliminary steps to their excretion by the kidneys, but we did not find them present in such cases. It is possible that traces were present, for we could not devise a practical procedure that would detect tartrates in a solution more dilute than one part in two thousand. It is also possible that the urinary excretion of tartrates might have occurred if the human dosage had been proportionally as large as some of the doses used in animal experimentation. The largest dose we

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used was 20 gm a day, which would surely seldom be exceeded in any diet or in the treatment of the sick. We were not trying to determine how much tartrate would need to be ingested to injure the kidneys, but rather to test the effect of quantities that might reasonably be prescribed in medical practice, either as medication or in the diet.

Tartrates, especially Rochelle salts, are pharmacologically classed both as saline cathartics and as diuretics. A cathartic action argues against much absorption, for the effectiveness of saline cathartics depends partly upon difficulty of absorption. On the other hand, it is not easy to see how an ingested salt can act as a diuretic unless it is absorbed from the intestine and carried by the blood to the kidneys. There is, however, another possible explanation for tartrate diuresis, as will be seen later in this discussion.

The alkalinizing effect of tartrates, which our former tests tended to prove, would be strong evidence of their oxidation in the tissues if their absorption from the intestine were known to occur, but the results of our recent experiments have convinced us that neither such absorption nor such oxidation are necessary in order to produce this effect. While we have, therefore, not found the final answer to the first two of the three questions stated above, we believe that they do not need to be answered in the affirmative.

The clue which we have followed in trying to answer the third question came from Simpson's<sup>6</sup> statement that a rabbit's intestinal contents can decompose tartrate ions in twenty-four hours and that a bouillon culture of *Escherichia communior* can do so in twelve days. In view of the important possibilities which such a finding opens up, it is surprising that no record of further work along this line has appeared in the literature since his report.

Three hours after a meal we gave one of our subjects 10 gm of magnesium sulphate. A fluid stool specimen was obtained. Three flasks were prepared, each containing 10 c c of the stool and 15 c c of water. In the first was placed 0.1 gm of Rochelle salts ( $\text{COOK-CHOH-CHOH-COONa}$ ), in the second 0.1 gm of potassium acid tartrate ( $\text{COOK-CHOH-CHOH-COOH}$ ), and the third 0.1 gm of tartaric acid ( $\text{COOH-CHOH-CHOH-COOH}$ ). All three flasks were incubated at  $37^\circ \text{C}$  for twenty-four hours and tests made for the presence and quantity of tartrates. Results are shown in Table I.

TABLE I

FLASK 1	FLASK 2	FLASK 3
No tartrate present	0.04 gm tartrate	0.07 gm tartrate

A 5 gm portion of a normal stool specimen from the second subject was emulsified with 25 c c of water and 0.3 gm of Rochelle salts. After twenty-four hours' incubation at  $37^\circ \text{C}$  no tartrate could be recovered.

Normal stool specimens were obtained from the third and the fourth subjects. Three series of five flasks each were prepared, each flask containing 25 c c of water and 0.2 gm of Rochelle salts. In each flask of the first series we placed 2 gm of the third subject's stool, in each flask of the second series 5 gm of the third subject's stool and in each flask of the third series 5 gm of the fourth subject's stool. The flasks were incubated for different lengths of time. Our plan was to get some idea of the rate at which different quantities of feces would decompose tartrate ions. In-

incubation periods and tartrate losses in approximate percentages are given in Table II

TABLE II

SERIES	FLASK 1 2 HOURS	FLASK 2 3 HOURS	FLASK 3 4 HOURS	FLASK 4 6 HOURS	FLASK 5 24 HOURS
1	10% loss	20% loss	35% loss	50% loss	100% loss
2	40% loss	50% loss	60% loss	80% loss	100% loss
3	50% loss	70% loss	90% loss	100% loss	100% loss

We followed this line of investigation further, using stool specimens sent to our clinical laboratory for analysis. In each case approximately 5 gm. of the stool was put into a flask with 50 c.c. of water and 0.2 gm. of the tartrate being tested at the time. We prepared 60 flasks, using Rochelle salts, and 50 using potassium acid tartrate. All were incubated at 37° C. for twenty-four hours and the contents of the flasks tested for tartrates. One hundred and seven of the 110 flasks showed no tartrates at all. Two showed heavy traces. One showed about 0.1 gm. For the two flasks we had purposely chosen stool specimens that were hard and dry and about a week old when tested. That used in the one flask consisted of a mixture of unmasticated, sliced, raw onions and yellowish mucus, passed by a toothless old lady who had developed a diarrhea after indulging a perverted appetite a bit too far.

The above findings show that the normal human intestinal contents are capable of decomposing tartrates at a rate which would insure the destruction of all the tartrations in a U. S. P. dose of Rochelle salts or potassium acid tartrate in much less than the time food normally remains in the absorbing part of the intestine. Catharsis might hasten the passage of food along the digestive tract enough to cause this decomposition to be incomplete, but in any case it should take place to a considerable extent.

Table I shows that the less acid the tartrates are the more easily they are broken down. Normally, the intestinal contents possess sufficient alkalinity to neutralize all the potassium acid tartrate that would ordinarily be ingested, and tartaric acid, as such, is seldom taken into the human stomach in appreciable amounts. The normal physiologic condition of the intestine, therefore, would favor the process of tartrate decomposition.

Further tests were made to determine what constituent or constituents of the feces might be responsible for the observed decomposition. These tests are described below in sufficient detail to make the purpose and procedures clear.

Thirty cubic centimeters of human bile was collected by means of a duodenal tube. Ten cubic centimeters was placed in each of three flasks, along with 0.2 gm. of Rochelle salts and 40 c.c. of water. The three flasks were incubated for five days without loss of tartrates.

Into each of five flasks we placed 1 gm. of pancreatin, 0.2 gm. of Rochelle salts and 50 c.c. of water. These flasks were also incubated for five days. No loss of tartrates could be detected in any of the flasks.

Ninety flasks were prepared and divided into three lots of thirty each, each flask containing 50 c.c. of plain nutrient broth. One lot was inoculated with *Escherichia coli*, one with *Escherichia communior*, and one with *Escherichia acid-lactici*. All were incubated for twenty-four hours at 37° C. Into each flask we then put 0.2 gm. of Rochelle salts and incubated all of them for twenty-four hours more.

Those inoculated with *E. coli* and *E. communior* showed complete decomposition of tartrates. The first few of the flasks tested that had been inoculated with *E. acidilactici* showed heavy traces of tartrates still undecomposed. After another twenty-four hours of incubation of the remainder of the flasks, the decomposition was found to be complete.

We next took 50 gm. portions of seventeen different stools that came into the clinical laboratory. Each portion was emulsified with ten times its weight of water and filtered through a Berkefeld filter. Enough filtrate was obtained to give 50 c.c. for each of 78 flasks. The first ten were inoculated with *E. coli*, the second ten with *E. communior* and the third ten with *E. acidilactici*. All the flasks were incubated at 37° C. for twenty-four hours, 0.2 gm. of Rochelle salts was added to each flask, and the incubation continued for forty-eight hours more. No tartrates could be recovered from any of the thirty inoculated flasks, but there seemed to be no loss of tartrates in the remaining 48 uninoculated flasks.

The four tests just described indicate that the decomposition of tartrates by feces is not due to chemicals or enzymes *per se*, but rather to the action of living bacteria, particularly to that of the two common varieties of colon bacillus. We made further tests, however, to see if an alkalizing effect could be produced by such bacterial action outside the human body.

Twelve pour plates were prepared, using plain nutrient agar to which a little brom thymol blue and 1 per cent of Rochelle salts had been added and the  $P_H$  adjusted to 6.4, giving a greenish-yellow tint. Four of the plates were streaked with *E. coli*, four with *E. communior*, and four were left sterile for controls. All were incubated at 37° C. After twenty-four hours there was a distinct blue halo about the colonies of bacteria on each of the inoculated plates, showing that alkali had appeared as a result of the bacterial action. After forty-eight hours the blue color had spread throughout the agar. The color in the sterile plates had changed very little, at most enough to indicate a shift of  $P_H$  from 6.4 to 6.6.

Fifty cubic centimeters of plain nutrient broth was put into each of twelve flasks. All were inoculated with *E. coli* after the  $P_H$  had been adjusted to 7.6. Three tenths cubic centimeter of alcoholic phenolphthalein solution was then dropped into each flask and 0.2 gm. of Rochelle salts was put into each of six of the flasks. All were incubated in the usual way. Within forty-eight hours a pink tinge appeared in four of the flasks to which Rochelle salts had been added, and after twenty-four hours more each of these six flasks showed a distinct light reddish-brown color. The color of the broth in the six flasks to which no Rochelle salts had been added showed no such change, though the broth became cloudy from the growth of bacteria.

We cannot prove the nature of the tartrate decomposition which occurred in these tests. It is likely, however, that carbon dioxide and water were formed. If so, whenever Rochelle salts was the tartrate used, both sodium and potassium bicarbonates would be among the decomposition products. These could produce the observed alkalizing effect. Furthermore, if such a series of chemical reactions occurred in the human intestine, these bicarbonates would readily be absorbed and could easily cause the mild diarrhea sometimes noticed after the ingestion of tartrates.

As a test for bicarbonates, we added a few drops of strong hydrochloric acid to

each of the twelve flasks used in the last test mentioned above, after the seventy-two hours' incubation. A few very small bubbles of gas arose from each, as near as we could estimate about three or four times as many from the flasks to which Rochelle salts had been added as from the control flasks, but we could not call what we saw an unmistakable effervescence. We have evidence, therefore, but it is not conclusive enough to amount to proof, that bicarbonates were formed as a result of bacterial action on the Rochelle salts in these test flasks.

Our conclusion from the two tests last described is that the common fecal bacteria are in the course of their growth able to decompose tartrates, and thereby increase the alkalinity of the medium in which this growth and decomposition are taking place. If, as we feel justified in believing, the acid radicals of ingested alkali tartrates are largely or wholly broken down in the human intestine by bacterial action, we have a logical explanation of all our findings. First, the decomposition of tartrate ions would set free easily absorbable sodium and potassium ions, which could produce the observed alkalinizing effect. Second, if the tartrate ions were decomposed before absorption, we should expect to see exactly what we did see, no signs of kidney damage and no tartrates in the urine. Third, the occasional mild diuresis observed could have been due to the decomposition products of tartrates, probably bicarbonates, rather than to the tartrates themselves.

#### SUMMARY

1 In our previous work we found ingested tartrates to be alkalinizing in the human body, but the method by which this effect is produced was not settled.

2 We have recently given attention to three questions: (1) Are tartrates absorbed from the human intestine? (2) If absorbed, are they oxidized in the tissues? (3) If not absorbed and oxidized, how can they be alkalinizing?

3 If tartrates are absorbed, they are oxidized in the tissues, for the human kidney does not excrete more than traces of tartrates, if any at all, after their ingestion.

4 Tartrates are decomposed by feces and by the common fecal bacteria, but not by bile, pancreatin or Berkefeld filtrates of feces. The ease and rapidity with which this decomposition takes place shows that at least the major portion of ingested tartrates can normally be decomposed in the lumen of the intestine.

5 In the process of decomposition of the alkali salts of tartaric acid by cultures of fecal organisms, the medium in which the decomposition takes place is made more alkaline. It is, therefore, not necessary for tartrates to be absorbed from the intestine and oxidized in the tissues in order to exert their characteristic influence upon the human body. The absorption of the decomposition products can account for the alkalinizing effect which was uniformly observed, as well as the mild diuresis which was occasionally noticed.

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# A STUDY OF THE OCCURRENCE OF UNFERTILIZED ASCARIS EGGS\*

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THE diagnosis of the intestinal helminths which are commonly found in man can be made readily by any one of the numerous methods of fecal examination. Most of the eggs which are found in the feces are characteristic for each worm. In the case of *Ascaris lumbricoides*, however, both fertilized and unfertilized eggs may be found. This fact introduces a possible source of error in the diagnosis of this particular parasite. There is not very much information available concerning the frequency with which fertilized and unfertilized *Ascaris* eggs occur in cases of ascariasis.

Fertilized eggs are very characteristic, having a thick, clear, inner shell covered by an irregular, warty, albuminous coat which is stained yellow or brown in the intestine. They are usually spherical or slightly oval in shape and measure from 45 to 75 micra in length by 35 to 50 micra in width. Unfertilized eggs are much longer, narrower, more elliptical in shape. They measure from 70 to 88 micra in length by 38 to 50 micra in width. The inner structure is unorganized and frequently contains amorphous granular material or it may be vacuolated. The albuminous coat may be absent from both the fertilized and unfertilized eggs. The unfertilized eggs are usually bile stained but are more likely to be colorless, gray, or unstained than are fertilized eggs. Fig 1 shows both fertilized and unfertilized *Ascaris* eggs. These illustrations were furnished by and are used with the permission of Doctors G F Otto of the Department of Helminthology of the Johns Hopkins School of Hygiene and Public Health and John E Stumber of the Rockefeller Institute for Medical Research.

This study was undertaken to ascertain the following points: (a) the frequency with which unfertilized *Ascaris* eggs occur alone in fecal specimens, (b) the frequency with which fertilized *Ascaris* eggs occur alone, (c) the frequency with which both kinds of *Ascaris* eggs appear, (d) the relative proportion of fertilized and unfertilized eggs in cases where both kinds were present in the specimen.

In making this differential *Ascaris* egg count study a series of 1,103 specimens of feces which contained *Ascaris* eggs were used. A single egg-count on each specimen was made, using the Stoll egg-counting method. By using this method the intensity of infestation was determined and the approximate percentage of fertilized eggs in each specimen was recorded according to Table I which shows the relative proportion of fertilized eggs estimated to be present in each specimen.

Group 1 contains no fertilized eggs or unfertilized eggs alone. The percentage of fertilized eggs increases until group 8 is reached in which 100 per cent of the eggs are fertilized.

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All of the egg-counts were done by two technicians who were well trained both in the diagnosis of these helminths and in the use of the Stoll egg-counting technic. The specimens were obtained from school children in the eastern and middle sections of Tennessee. The average intensity of infestation for the entire series was 108,300 *Ascaris* eggs per gram of feces.

Table II shows the frequency of the two kinds of *Ascaris* eggs in this series. In 26.2 per cent of the specimens unfertilized eggs alone were present while in 32.2 per cent of the specimens all of the eggs were fertilized. In 41.6 per cent

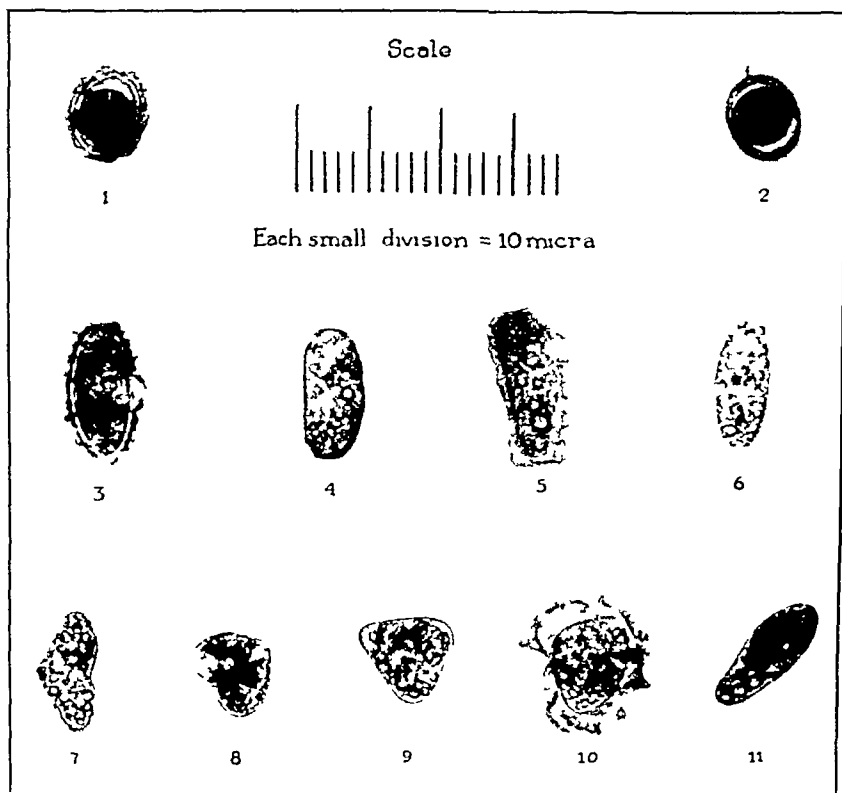


Fig 1—Fertilized and unfertilized *Ascaris* eggs. No 1 and 2 typical fertilized eggs with albuminous capsule. Nos 3, 4, 5 and 10 unfertilized eggs with albuminous capsule. Nos 6, 7, 8, 9 and 11 unfertilized eggs without albuminous capsule.

both fertilized and unfertilized eggs were noted. It can be seen that unfertilized eggs alone were found almost as frequently as fertilized eggs.

In determining the relationship between the intensity of infestation and the percentage of fertilized eggs, the following intensity grouping was used:

By using this classification it is possible to determine approximately the total number of worms present as it is estimated that there is one *Ascaris* present for every 1,000 eggs per gram of feces. It is also considered that there is an equal number of male and female worms.

Table IV shows the distribution of fertilized eggs according to the above intensity grouping. By computing the arithmetic mean in each intensity group the average percentage of fertilized eggs can be determined.

It can be seen from this analysis that the percentage of fertilized eggs increases with the intensity of infestation. It is also interesting to note that in the 289 cases in which unfertilized eggs occurred alone 26.5 of these or 91.7 per cent had light infestations, that is five female worms or less. This is the group in

TABLE I  
ESTIMATED PER CENT OF FERTILIZED EGGS IN EACH SPECIMEN

GROUP	PER CENT FERTILIZED EGGS	GROUP	PER CENT FERTILIZED EGGS
1	0	5	60
2	15	6	75
3	35	7	90
4	50	8	100

TABLE II  
PER CENT OF FERTILIZED AND UNFERTILIZED ASCARIS EGGS IN 1103 SPECIMENS OF FECES

TYPE OF EGG	NUMBER OF SPECIMEN	PER CENT
Unfertilized	289	26.2
Fertilized	355	32.2
Unfertilized and fertilized	459	41.6
Total	1103	100.0

which the diagnosis is most likely to be missed if unfertilized *Ascaris* eggs are not recognized. In those very light infestations in which only unfertilized eggs were present, there was an average of 2,300 eggs per gram of feces. In those specimens containing only fertilized eggs there was an average of 4,500 eggs per

TABLE III  
CLASSIFICATION OF INTENSITY OF INFESTATION OF ASCARIS

INTENSITY OF INFESTATION EGGS PER GRAM OF FECES	APPROXIMATE NUMBER OF FEMALE WORMS	CLINICAL CLASSIFICATION
0-9,999	0-5	Very light
10,000-49,999	6-25	Light
50,000-99,999	26-50	Moderate
100,000-299,999	51-150	Heavy
300,000 and over	151 and over	Very heavy

TABLE IV  
INTENSITY OF INFESTATION, PERCENTAGE OF FERTILIZED EGGS IN EACH GROUP

INTENSITY OF INFESTATION ESTIMATED NUMBER OF FEMALE ASCARIDS	PER CENT OF FERTILIZED EGGS								TOTAL NO OF SPEC	AVERAGE PER CENT FERTILIZED EGGS
	0% NO OF SPEC	15% NO OF SPEC	35% NO OF SPEC	50% NO OF SPEC	60% NO OF SPEC	75% NO OF SPEC	90% NO OF SPEC	100% NO OF SPEC		
0-5	265	5	25	24	20	24	13	178	554	41.8
6-25	24	15	20	21	29	55	92	138	394	76.0
26-50	0	8	4	6	6	14	42	25	105	78.5
51-150	0	1	1	1	2	5	20	14	44	86.2
151 and over	0	0	0	0	0	0	1	5	6	98.3
Total	289	29	50	52	57	99	172	355	1103	

gram of feces. When there are more than five female worms present, there is a progressive increase in the number of fertilized eggs.

#### DISCUSSION

The diagnosis of *Ascaris lumbricoides* by the examination of feces is not difficult when fertilized eggs are present in the specimen. The fact that not only fertilized but also unfertilized eggs may be found either alone or in the same specimen in ascariasis introduces a possible error in the diagnosis of this condition. If unfertilized *Ascaris* eggs are unrecognized, the degree of error may be large as in this series, 26.2 per cent of the specimens contained unfertilized eggs alone. In diagnostic laboratories where routine stool examinations are made, the diagnosis may be missed if these eggs are not recognized. In making extensive intestinal parasite studies, the information concerning the prevalence of *Ascaris* may not be accurate if this factor is not taken into consideration.

#### SUMMARY

1. A differential egg count analysis was made on 1,103 specimens containing eggs of *Ascaris lumbricoides*.
2. In this series 26.2 per cent of the specimens contained unfertilized *Ascaris* eggs alone, 32.2 per cent contained fertilized eggs alone and 41.6 per cent contained both fertilized and unfertilized eggs.
3. The relative percentage of fertilized *Ascaris* eggs increases with the increase in the intensity of infestation.
4. The smallest number of fertilized eggs were found in the very light infestations, one to five female worms. When more than five female worms are present there is a progressive increase in the percentage of fertilized eggs.

## A STUDY OF FROHDE'S TEST FOR MORPHINE\*

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FROHDE'S test, which depends upon the reduction of a molybdate in acid solution, is one of the earliest and most used tests for morphine. With this alkaloid, Frohde's reagent gives a reddish violet† color soon changing to blue, then to green, yellow, and light red. The test was first proposed by Frohde in 1866,<sup>1</sup> and as a test with the alkaloids has been used frequently by various investigators.

Witthaus<sup>2</sup> lists a number of alkaloids and glucosides which give a red or violet color with this reagent, but states that all except toxopleiygin give the same color with the sulphuric acid alone. Rojahn and Staufmann<sup>3</sup> tried a number of alkaloids and glucosides with Frohde's reagent. They found that arbutine and strophanthine give a violet coloration.

Miller and Taylor<sup>4</sup> and Levine and Yah<sup>5</sup> tested a number of organic compounds by heating them with a dilute solution of a molybdate and sulphuric acid. A number of these gave color reactions. However, in the regular test for morphine with Frohde's reagent no heat is used, and the reagent contains no water.

The object of the investigation reported in this paper was to test a large number of organic compounds with Frohde's reagent in order to determine whether or not there were any great number of substances which would give the morphine test, and whether or not this test was characteristic of any given organic grouping, or class of organic compounds. Also, these organic compounds were mixed in varying amounts with morphine, and the test applied in order to determine which substances would interfere with the test.

### PROCEDURE

The organic compounds numbering over 450, and the methods used were approximately the same as those employed in a similar study of strychnine.\*

*Preparation of Frohde's Reagent* One gram of finely ground sodium molybdate was dissolved in 100 c.c. of chemically pure concentrated sulphuric acid with the aid of heat.

*Preparation of Standard Morphine Solution* A solution was prepared in alcohol containing one milligram of morphine per cubic centimeter.

*Preparation of Solutions to be Tested* The compounds to be tested were dissolved in the proper solvent so that one cubic centimeter of the solution was equivalent to one milligram of the compound. Care was taken in selecting a suitable solvent which would not only readily dissolve the compound, but would also volatilize on the water bath. Substances which were relatively insoluble were finely ground and suspended in some volatile solvent. The bottle was shaken before removing a portion of the contents to insure an even suspension of the substance to be tested.

*Procedure of Tests* Three tests were made on each compound. For the first test, one cubic centimeter of the solution to be tested was evaporated to dryness and then tested with three or four drops of Frohde's reagent, and any color change was recorded. In the second

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†Most text books describe the color as purple or violet.

test, one cubic centimeter of the solution to be tested and one cubic centimeter of standard morphine solution were mixed and the test made as described above. If there was no interference, a reddish violet color resulted which soon changed to green. If there was an interference in the color reaction, the colors resulting were recorded. The third test was carried out as above, except that five cubic centimeters of the solution of the organic compound were used with one cubic centimeter of the morphine solution.

The results of the tests when the organic compounds were treated with the reagent are given in the pages immediately following. The name of the organic compound is given first, and directly opposite is given the color reaction. In cases where no color was developed, the compounds are listed with the statement, "No color reaction."

#### ALKALOIDS AND ALKALOIDAL SALTS

ORGANIC SUBSTANCES	COLOR REACTION
Aconitine	Blue to green
Apomorphine	Green to blue, trace violet
Belladonnine	Brown
Berberine	Dirty brown
Brucine	Reddish violet to blue
Codeine	Green to blue
Colchicine	Brownish orange
Cotarnine hydrochloride	Brick red
Cryptopine	Violet to green
Delphinine	Brown, purple streaks
Dionine	Greenish yellow
Emetine	Brown, trace violet
Heroin	Reddish violet
Hydrastine	Green to brown
Morphine	Reddish violet to green
Narceine	Brown to green
Papaverine	Light violet to green
Phenacaine	Light green
Piperine	Reddish orange to brown
Quinine	Pale green
Sanguinarine nitrate	Brown, trace purple
Solanine	Yellow
Veratrine	Yellow to orange

*No Color Reaction.*—Atropine, caffeine, cinchonidine, cinchonine, cocaine, daturine, ergotamine, beta eucaine hydrochloride, gelseminine, homatropine, hyoseyamine, nicotine, pelletierine, physostigmine, pilocarpine, pseudopelletierine, quinidine, scopolamine, sparteine, strychnine, theobromine, theophylline

#### AMINO ACIDS AND DERIVATIVES

para Aminophenylglycine	Dull green
Aspartic acid	Tan
Duodotyrosine	Greenish grey
Edestine	Yellow
Glycyltryptophane	Greenish orange
para Nitrophenylglycine	Brown
Phenylglycine	Brown
Tryptophane	Dirty green
Tyrosine	Blue

*No Color Reaction.*—Acetylphenylglycine, alpha alanine, arginine, asparagine, di benzoyl alanine, betaine hydrochloride, creatine, creatinine, ethylglycollate, glutamic acid, glycine, hypuric acid, isoleucine, leucine, alpha phenylalanine, beta phenylalanine, di valine

## ALIPHATIC ACIDS

Palmitic acid

Dirty brown

*No Color Reaction*—Aconitic acid, adipic acid, dl alpha aminoacetylacetic acid, alpha bromopropionic acid, beta bromopropionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mucic acid, propionic acid, stearic acid, succinic acid, tartaric acid, tartromic acid, trichloroacetic acid

## ALIPHATIC ACID SALTS, ESTERS, AND DERIVATIVES

*No Color Reaction*—Ethyl oxalate, ethyl succinate, isoamyl propionate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate, thallous formate, thallous malonate, triacetin, tributyrin

## ALIPHATIC ALCOHOLS AND KETONES

Methyl heptenone

Dirty yellow

Phorone

Tan

*No Color Reaction*—Acetylacetone, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isobutyl alcohol, isopropyl alcohol, mannitol, melissyl alcohol, octyl alcohol, trichlorobutyl alcohol.

## SUGARS

Levulose

Light yellow

Melezitose

Pale orange

Xylose

Grey

*No Color Reaction*—Arabinose, galactose, glucose, lactose, maltose, d mannose, raffinose, rhamnose, sucrose

## UREA AND URIC ACID DERIVATIVES

Allylphenylthiocarbamide

Yellow

*No Color Reaction*—Allylthiocarbamide, allylthiourea, amytal (isoamylethylbarbituric acid), acetylmethylurea, allantoin, alloxantine, barbital (diethylmalonylurea), barbituric acid, biuret, dl n butylthiourea, dibromobarbituric acid, diphenylthiourea, guanine hydrochloride, ipral (calcium ethylisopropylbarbiturate), luminol (phenylethylbarbituric acid), peralga (aminopyrimethethylbarbiturate), thiobarbituric acid, thiourea, triphenylguanidine, urea, urethane, uric acid

## GLUCOSIDES

Arbutin

Violet

Colocynthin

Reddish brown to purple

Convallamarin

Brown

Digitalin

Magenta, streaks of purple

Elatarin

Olive green

Phloridzin

Navy blue

Picrotoxin

Yellowish orange

Salicin

Violet blue to rose

Saponin

Greenish blue, streaks of violet

*No Color Reaction*—Aesculin, amygdalin, santonin

## MISCELLANEOUS ALIPHATIC COMPOUNDS

Amnoguanidine bicarbonate

Dirty green

Bromoform

Greenish yellow

Isobutylbromide

Slight yellow

Oxamide

Grey

*No Color Reaction*—Acetal, acetaldoxime, acetamide, acetoxime, aldehydeammonia, tertiary butyl bromide, chitin, chloral hydrate, chloral urethane, chloropicrine, dimethylglyoxime, hexachloroethane, hexamethylenetetramine, iodoform, methylglyoxal sodium bisulphite, monochlorohydrine, nitrosodiethylamine, oenanthol, pinacol hydrate, propionamide, sulphonol, thialdime, tribromohydrine, trichloroacetylchloride, trimethylene bromide, trional, veronal

#### BENZENE AND TOLUENE DERIVATIVES

Azobenzene	Deep yellow
Mesitylene	Yellowish brown

*No Color Reaction*—ortho Bromochlorobenzene, para bromochlorobenzene, ortho bromonitrobenzene, meta chloronitrobenzene, ortho chloronitrobenzene, para chloronitrobenzene, para chlorotoluene, ortho dichlorobenzene, 2,5 dichloronitrobenzene, iodosobenzene, isopropylbenzene, meta nitrotoluene, ortho nitrotoluene, para nitrotoluene, styrene, para xylenesulphonic acid

#### ANILINE AND DERIVATIVES

Acetyl ortho methyltoluidine	Greenish yellow
Aminoazobenzene	Dark brown
meta Anisidine	Grey
ortho Anisidine	Grey
para Anisidine	Blue to grey
meta Bromoaniline	Brown, traces of violet
ortho Phenetidine	Grey, streaks dirty purple
para Phenetidine	Grey, streaks dirty purple
ortho Toluidine	Brown
meta Toluidine	Greenish brown
para Toluidine	Brown

*No Color Reaction*—Acetamide, acetyl para anisidine, acetyl para methyltoluidine, acetylphenetidine, acetyl ortho toluidine, acetyl para toluidine, meta acetylidine, 2 amino toluene 4 sulphonic acid, 2 aminotoluene 5 sulphonic acid, 4 aminotoluene 2 sulphonic acid, aniline, benzamide, ortho benztoluide, para benztoluide, para bromoacetamide, ortho bromo aniline, para bromoaniline, chloramine, meta chloroaniline, ortho chloroaniline, para chloro aniline, 1,2,4 dichloroaniline, 2,5 dichloroaniline, 1,2,4 dinitroaniline, evulgine, meta nitro aniline, para nitroaniline, meta nitrodimethylamine, para nitrodimethylamine, nitrosodimethyl aniline, 1,2,3 nitrotoluidine, 1,2,4 nitrotoluidine, 1,3,4 nitrotoluidine, phenyl beta diphenyl amine, ortho toluidine, tribromoaniline, trinitroaniline, 1,2,4 xylidine, 1,3,4 xylidine

#### PHENOLS AND DERIVATIVES

Acetyl para aminophenol	Pale blue
ortho Aminophenol	Reddish violet
para Aminophenol	Dirty green
5 Benzylamino 2 cresol	Bright green
para Benzylaminophenol	Light green
Benzoylthymol	Greenish rose
Carvacrol	Brownish green, streaks violet
Catechol	Grey
ortho Chloromercurephenol	Bluish green
Phloroglucinol	Tan
Pyrogallie acid	Black, streaks violet

*No Color Reaction*—Acetyl meta aminophenol, meta aminophenol, para bromophenol, ortho chlorophenol, para chlorophenol, meta cresol, ortho cresol, para cresol, 3,5 dibromo ortho cresol, 2,4 dichlorophenol, dimethylhydroresorcinol, 2,3 dinitrophenol, 2,4 dinitrophenol, 2,6 dinitrophenol, meta nitrophenol, ortho nitrophenol, para nitrophenol, orcinol, phenol, picric acid, tetrabromo ortho phenol, thymol, tribromophenol, trichlorophenol, xylenol



## AROMATIC ACIDS

Benzilic acid	Rose, trace of purple
Cinnamic acid	Yellowish green
Cumaric acid	Green to grey
Gallie acid	Green
Tannic acid	Brownish green

*No Color Reaction*—Acetylsalicylic acid, meta aminobenzoic acid, para aminobenzoic acid, diaminophenylacetic acid, anisic acid, anthranilic acid, arsanilic acid, meta bromobenzoic acid, ortho bromobenzoic acid, para bromobenzoic acid, meta chlorobenzoic acid, ortho chlorobenzoic acid, para chlorobenzoic acid, cinchophen, diiodosalicylic acid, diphenylacetic acid, 5 iodosalicylic acid, mandelic acid, para mercurichlorobenzoic acid, metanilic acid, naphthionic acid, meta-nitrobenzoic acid, ortho nitrobenzoic acid, para nitrobenzoic acid, quinic acid, salicylic acid, terephthalic acid, ortho toluic acid, para toluic acid

## AROMATIC ACID DERIVATIVES

Methyl cinnamic ester	Greenish blue
Neocinchophen	Yellowish green
(ethyl 6 methyl 2 phenylquinoline 4 carboxylate)	
Phenyl salicylate	Greenish grey

*No Color Reaction*—Benzamide, benzoic anhydride, benzyl benzoate, butyl benzoate, coumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, isoamyl salicylate, isobutyl benzoate, methyl benzoate, methyl salicylate, nicotinic acid nitrate, meta nitrobenzoyl chloride, para nitrobenzoyl chloride, phthalimide

## AROMATIC ALDEHYDES, ETHERS, ALCOHOLS, AND KETONES

Anisaldehyde	Dirty green
Benzhydrol	Reddish orange
1, 2, 5 Bromosalicylaldehyde	Greenish yellow
ortho Nitrobenzaldehyde	Greenish yellow
Phthalic acid aldehyde	Tan
Salicylaldehyde methyl ether	Brown
Saligenin	Violet to blue
para Tolylaldehyde	Black with violet
Vanillin	Greenish yellow

*No Color Reaction*—para Aminoacetophenone, anisole, benzalacetone, benzalacetophenone, benzophenone, ortho bromoanisole, para bromoanisole, ortho bromonitrobenzaldehyde, ortho chlorobenzaldehyde, isophthalaldehyde, meta methoxysalicylaldehyde, methylacetophenone, para nitroanisole, 5 nitrosalicylaldehyde, phenetole, piperonal, salicylaldehyde, tetramethyldi aminobenzophenone

## HETEROCYCLIC COMPOUNDS

Acridine	Yellowish green
Furoic acid	Pink
Isatin	Orange

*No Color Reaction*.—Antipyrine, dimethylpyrone, 6 nitroquinoline, oxyquinoline, piperidine, quinaldine, quinoline, skatole, succinimide

## HYDROAROMATIC COMPOUNDS

Circene	Yellowish brown
Isomonene	Brownish black
Terpenyl acetate	Tan

*No Color Reaction*—d Borneol, l borneol, dl camphor (natural), camphor (synthetic), camphoric acid, camphorsulphonic acid, circenone, menthol, quercite, terpineol

## NAPHTHALENE AND ANTHRACENE DERIVATIVES

Acet alpha naphthalide	Greenish grey
Acet beta naphthalide	Greenish brown
Alizarin	Magenta, trace of purple
Dibromoanthracene	Light green
alpha Naphthylamine	Bluish green
alpha Naphthylaminorobenzene	Black with violet
alpha Naphthylisocyanate	Olive green

*No Color Reaction.*—Acenaphthene, alpha bromonaphthalene, 1,5 dinitronaphthalene, beta naphthalenesulphonic acid, naphthylidene anhydride, beta naphthol, naphtholmethyl alpha ether, beta naphthylamine, nitroso beta naphthol

## MISCELLANEOUS AROMATIC COMPOUNDS

Abietic acid	Brown
Adrenaline	Blue to green
Amarine	Bright green
Benzcatechin	Greenish brown, trace of purple
Benzoin	Pink
Benzylphenylhydrazine	Grey blue to violet
Isoniylphenylhydrazine	Greenish grey
Isoeugenole	Brown black
Phenanthrene	Deep orange
Phenanthrenequinone dioxide	Brown, streaks of purple
Phenolphthalein	Reddish orange
meta Phenylenediamine hydrochloride	Greenish blue
Phenylhydrazine hydrochloride	Light green
Rheumatin (saloquinne salicylate)	Yellowish green
Salvarsin	Green
Tetrabromophenolphthalein	Rose
Thymolphthalein	Magenta
Triphenylmethane	Bluish green
Turmeric	Brownish purple

*No Color Reaction.*—Benzil, 2,4 dinitrophenylhydrazine, diphenyl, fluorene, ortho nitro acet meta xylidide, meta nitrobenzhydrazide, para nitrophenylhydrazine, quersite, thiosemi carbazide, ortho tolunitrile, para tolunitrile, para tolylthiocyanate, para tolylquinoline sulphate, para tolylthioquinanthrene

The color reactions for the tests where an equal amount of impurity was added to the morphine were recorded, and also the reactions where five times the amount of impurity was added, but the listing of these would require too much space. Therefore, only those reactions in which the reddish-violet color was completely masked will be given. The list of substances previously given may be referred to in order to obtain the names of the compounds which did not completely cover up the morphine test. Many of these gave no interference, but others gave more or less interference.

Organic compounds which completely covered up the morphine test when present in equal amounts were Azoxybenzene, para-aminoazobenzene, adienaline, benzalacetophenone, and nicotinic acid nitrate.

Organic compounds which completely covered up the morphine test when present in amounts five times that of morphine were Azoxybenzene, para-aminoazobenzene, para-aminophenol, adienaline, benzalacetophenone, benzhydrol, beta-

naphthylamine hydrochloride, nitrosodiethylamine, nicotinic acid nitrate, piperine, phenolphthalein, and sanguanine nitrate

A study of the preceding data shows that there were twenty-nine compounds which gave various shades of violet, lavender, or purple, which might, more or less, be confused with the Frohde's test for morphine. However, over a fourth of the compounds gave some color test with the reagent. In general, the compounds giving a similar color, as does morphine, with this reagent do not belong to any definite group of organic compounds, nor does any special organic radical seem to be responsible for the characteristic test. As a group, the alkaloids seem to give more of the positive test than does any other group.

There were very few organic compounds which completely masked the morphine test. However, there were many interferences to a greater or less degree. Of course, in a carefully conducted examination for morphine many of the foreign organic compounds would be removed by means of different organic solvents.

#### CONCLUSIONS

1 A number of organic compounds have been found to give a color test similar to that given by morphine with Frohde's reagent.

2 The interferences of organic compounds with Frohde's test for morphine have been determined when present in varying amounts.

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### THE LOCAL IMMUNIZATION OF THE PERITONEUM BY THE USE OF BACTERIOPHAGE A NEGATIVE REPORT\*

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IN THE course of a long series (approximately 500) of suppurative cases treated by local application of bacteriophage under the direction of the author<sup>1</sup> it was found that appendiceal and other abscesses in the abdominal cavity, due to *Staphylococcus aureus* and *Escherichia coli* did very well after irrigation with bacteriophage filtrates. Some of our best results were those with persistent abscess cavities discharging large amounts of pus. In nearly every such case the cavity rapidly cleaned itself and began to heal rapidly. In a few cases which were particularly suitable for such study and in which the patient had a low blood leucocyte count there was a marked increase in the number of white cells in the circulating blood,

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<sup>1</sup>*Rice, Am J M Sc* **179** 345, 1930.

after the local administration of bacteriophage by instillation into the abscess cavity

Inasmuch as it is better to prevent infection than attempt to treat it, the thought came to us that it might be possible to immunize the peritoneum by injecting into the peritoneal cavity a mixture of staphylococcus—streptococcus—*B. coli* bacteriophage filtrates. Such a procedure if successful would apparently be of much value as a prophylactic measure to precede an abdominal operation likely to result in contamination of the peritoneal cavity. Experiments upon man being manifestly out of the question, the dog was chosen as a suitable experimental animal.

It was easily possible to make preliminary experiments upon animals that were being used by the Senior class of Indiana University School of Medicine in the course in Operative Surgery on the dog under the direction of Dr. W. D. Little. These dogs were aseptically operated upon under ether anesthesia. After the required experiments were done the students were asked to tie off the cecum with sterile silk sutures before closing the abdomen. Half of the dogs had been given an injection of Staph. strep.-coli "phage" on the three days immediately preceding the operation while the others had had no experience with it. It seemed that the animals with "phage" did better than those without, but the operations in the hands of inexperienced students were not sufficiently accurate to enable us to make sure of our conclusions.

Five series of much more accurate experiments were planned to follow the preliminary tests just described. Thirty dogs were operated upon under the most careful aseptic technic that could be arranged. Half of these animals had been given bacteriophage intraperitoneally at some time preceding or during the operation, while the other half had had none. Dogs were chosen in pairs which were as much alike as possible in age, size, breed, and general condition. One of each pair was used as a test animal and the other as a control which was handled in every way like the test animal except with regard to the giving of the bacteriophage.

The skins of the dogs were washed thoroughly with soap and clean warm water, and then shaved or deprived of hair with a depilatory. The clean skin was then thoroughly scrubbed, dried with alcohol followed by ether and then painted with tincture of iodine which was later washed off with alcohol. A clean and freshly sterilized set of instruments was used on each animal. Sterile drapings were used in abundance and every possible precaution taken to prevent contamination. After closure the dogs were placed in a pen by themselves and watched closely. The first animals had their temperatures and white cell counts taken at frequent intervals but this was discontinued after the first twelve animals, as it did not seem to give information sufficiently valuable to compensate for the great amount of work in making these determinations. It seems that dogs vary widely in these respects and that a great mass of information would be needed to interpret such findings. All dogs were subjected to autopsy as soon as possible after death and those that lived were killed during or rather soon after convalescence so that the condition of the abdomen might be ascertained.

Original protocols are given

Series I—Twelve dogs, six tests and six controls. The test dogs in this experiment had been given 30 c.c. of mixed Staph. strep. coli phage on each of the three days preceding the operation and

then had the exposed cecum wrapped in a phage saturated gauze sponge for fifteen minutes at the time of operation, and had 15 c.c. of the phage poured over the cecum and into the abdominal cavity. At the time of operation one and one half inches of the empty cecum were tied off with sterile silk thread.

Dog II — (Test) Killed after five days while apparently very ill. Marked generalized peritonitis with much bloody exudate. Few adhesions, and little plastic exudate. Cecum completely gangrenous. No evidence of local abscess formation.

Dog I — (Control) Killed after five days while apparently in good condition, certainly better condition at least than the corresponding test animal. There was a large abscess in the abdominal wall just beneath the cecum (pus was brown in color and slimy). Purulent (not bloody) exudate in the abdominal cavity. Generalized peritonitis. Cecum gangrenous and with a definite abscess wall about it. Apparently the abscess had been ruptured (dog had jumped from a high table the day before). *Control in better condition than test animal.*

Dog III — (Test) Died after forty hours. Bloody exudate in peritoneum, some fibrinous exudate. No adhesions of consequence. Cecum not at all walled off, gangrenous and nearly digested.

Dog IV — (Control) Killed after five days while in fairly good condition. There was some evidence of peritonitis. The omentum was tightly adherent to everything in the region of the cecum. Upon tearing away these adhesions the cecum was found to be about the size of a hen's egg, considerably darker than the surrounding viscera, tight and distended, but apparently not gangrenous. There was no evidence of general peritonitis. Apparently nature had succeeded in preventing the spread of infection. *Control in much better condition than the test animal.*

Dog VI — (Test) Killed after fourteen days being at that time apparently in good condition. The omentum was found firmly adherent in many places. Loops of the bowel were also firmly joined. No evidence of active peritonitis at time of autopsy. In one place a well walled off abscess with thick creamy pus was found (1 c.c. of pus). This abscess was at the former site of the cecum as was proved by the fact that the black silk suture was found in the abscess cavity.

Dog V — (Control) Killed after fourteen days while apparently in good condition. Upon opening the abdomen there was no evidence of general peritoneal irritation. The omentum was adherent in only one or two places. The bowel loops were not adherent except in the region of the cecum. About the region of the cecum was a very tight knot of adhesions, but it was possible to make out the stump of that organ. The tied off portion was completely destroyed. There was no evidence of pus or recent active inflammation. *The control was in somewhat better condition than the test animal.*

Dog VII — (Test) Died after forty hours. The abdomen contained a large amount of bloody exudate. Cecum was gangrenous and ruptured. There was a small amount of plastic exudate on the bowels, but no effective walling off. Omentum badly congested, somewhat sticky but not tied up in adhesions.

Dog VIII — (Control) Killed at forty four hours. The peritoneal cavity contained no fluid exudate. Omentum rather firmly adherent about the cecum about which it forms a ball. Cecum was found tightly tied off but only slightly redder than the surrounding viscera. It was definitely not gangrenous. There was no abscess cavity about it and the remaining peritoneum was essentially normal in appearance. It was evident that the blood vessels from the omentum had succeeded in nourishing the tied off cecum. *Control very much better than the test animal.*

Dog X — (Test) Died after forty four hours. Abdomen contained a large amount of bloody exudate. Bowels greatly congested, and everywhere covered with a fibrinous exudate. The omentum covered the bowels and was rather well arranged about the cecum. There was a definite abscess cavity (beginning) about the gangrenous cecum.

Dog IX — (Control) Killed after forty four hours while apparently in fair condition. The peritoneum seemed comparatively normal. There was no exudate and no adhesions except about the cecum. In this region there was a bunch of massed adhesions showing little inflammation on the outside. By breaking down the friable adhesions we find exactly in the center of the mass the gangrenous but not ruptured cecum. *The control animal was in much better condition than the test animal.*

Dog XI — (Test) Killed after five days while apparently in normal condition. There was no evidence of peritonitis. Loops of bowel not at all adherent except in the region of the cecum.

where there was a mass the size of a lemon. The center of this mass contained the cecum which was not gangrenous and only a little darker than the surrounding normal bowel. Blood vessels of considerable size could be seen passing from the omentum to the tied off cecum. Apparently this animal would have lived.

Dog XII—(Control) Died after five days. Marked peritonitis with hemorrhagic exudate and fluid in the peritoneal cavity. Cecum gangrenous and practically digested. No evidence of walling off. *In this case the control was in much worse condition than the test animal.*

*Resume of results in Series I*—In five instances the test animal fared worse than the control. It seemed that the bacteriophage tended to prevent the plastic adhesions which would have sealed off the danger area. It seemed also to digest the devitalized tissue as is shown by the fact that the cecum in most of the test animals was markedly more disintegrated than in the controls. Bacteriophage was not able to control the peritonitis produced.

Criticism of this series might be made on the grounds that the bacteriophage was given in too large dosage and that it irritated the peritoneum previous to the operation. This was not the case so far as could be seen at the time of the operation, the two sets of animals being indistinguishable on this score. Various experimenters have shown that bacteriophage tends to attract leucocytes and to cause the rapid solution of suppurative tissue. This is probably the reason why infected areas liquefy and clean themselves so rapidly. It seems likely that this action prevented the formation of the protective adhesions which would have prevented spreading of the peritonitis.

Series II. Six dogs—Three tests and three controls. The test dogs had 30 c.c. of mixed Staph. strep. coli bacteriophage at the time of operation. The cecum was exposed, 3 to 5 c.c. of fecal material was pressed into it from the bowel. It was then tied off and then punctured. The fecal matter was pressed out into the peritoneal cavity in the region of the cecum but was not spread about. The hole in the cecum was then closed with purse string suture and turned in.

Dog XIV—(Test) Died after twenty four hours. There was a small amount of fluid exudate, moderate generalized peritonitis, no plastic exudate. Cecum was well closed, gangrenous, no adhesions about it.

Dog XIII—(Control) Killed after eight days while apparently in good condition. The omentum was slightly adherent to bowels and the bowels slightly adherent to each other in places except in region of the cecum where there was a ball about that organ. No evidence of pus or active peritonitis. *Control better than test.*

Dog XVI—(Test) Died after twenty four to thirty six hours. Moderate generalized peritonitis. Very small amount of fluid exudate, no plastic exudate. Cecum well closed, no adhesions about it.

Dog XV—(Control) Died after twenty four hours. Marked peritonitis, small amount of fluid exudate, moderate amount of plastic exudate. Cecum closed, no adhesions about it. *Control and test essentially the same.*

Dog XVIII—(Test) Died after twenty four hours. Marked generalized peritonitis. No fluid exudate, no plastic exudate, no adhesions. Cecum closed.

Dog XVII—(Control) Killed after eight days while apparently in good condition. There were signs of generalized peritonitis still somewhat active. There was an enormous mass of adhesions about the cecum. There was a pus cavity (2 to 3 ounces) in the region of the cecum but it was well walled off. *Control animal did considerably better than the test.*

*Resume of Series II*—Again the control animals recovered considerably better than those used as tests. Conclusions reached after Series I are strengthened. The bacteriophage seems to prevent walling off of the involved area but is unable to control the peritonitis.

Series III. Six dogs—Three test and three controls. The test animals were given mixed Staph. strep. coli bacteriophage (30 c.c.) three, two, and one days before operation, but none at the time of operation. The cecum was squeezed full of fecal material from the bowel and then tied one and one half inches from the end. It was not punctured.

Dog XIX—(Test) Died after seventy two hours. Marked generalized peritonitis, considerable quantity of fluid in the abdomen. Cecum gangrenous but not ruptured until manipulated.

at autopsy This animal had been closed at operation with difficulty and there was a large infected area in the line of closure This abscess contained a large amount of fluid pus and seemed in itself to be sufficient to cause death

Dog XX —(Control) Died after forty hours Very violent peritonitis Cecum gangrenous Much bloody exudate A few adhesions between omentum and bowel *Test animal fared some what better than the control*

Dog XXI —(Test) Died after seven days This animal seemed to be in good condition for several days after the operation and then changed for the worse and died At autopsy the wound was broken down Evidence of marked generalized peritonitis Cecum gangrenous and ruptured Small amount of plastic exudate at different places in the abdomen but little or no definite walling off

Dog XXII —(Control) Died after forty hours Very marked generalized peritonitis Large quantity of bloody exudate in the peritoneal cavity Omentum and bowel somewhat adherent but no definite walling off *Control worse than test*

Dog XXIII —(Test) Died after ninety hours Marked generalized peritonitis Copious bloody exudate, purulent Few adhesions were about the cecum which was gangrenous but there was no real walling off

Dog XXIV —(Control) Died after seventy hours Marked generalized peritonitis No adhesions of any consequence Considerable quantity of bloody purulent exudate Cecum was gangrenous *Control about like test*

*Resume of Series III* —In this series there was little difference between test and control though in each case the test animal lived longer Only in the case of Dog XXI was there, however, a significant difference Evidently the large mass of fecal material in the cecum made recovery nearly impossible

Series IV Six dogs—Three test and three controls In this series the test dogs were given 30 c c of mixed Staph strep coli bacteriophage on the third, second, and first day before operation, but none at operation The empty cecum (emptied by squeezing into the bowel) was tied off

Dog XXV —(Test) This dog was in good condition after thirteen days and was used by mistake for another operative purpose No autopsy was done but the animal was apparently doing well

Dog XXVI —(Control) Killed after twenty seven days while apparently in good condition Low grade peritonitis About 700 c c of thin bloody fluid found Mass of firm adhesions about cecum which had sloughed Omentum injected *Both did well*

Dog XXVII —(Test) Died after three days Considerable quantity of bloody fluid in peritoneal cavity Marked generalized peritonitis Cecum gangrenous and ruptured, not walled off, no adhesions

Dog XXVIII —(Control) Died after four days Marked generalized peritonitis with bloody purulent exudate Cecum was gangrenous and ruptured Some slight plastic exudate, no walling off *No real difference between test and control animals*

Dog XXX —(Test) Died after three days Thick bloody purulent exudate in peritoneal cavity No plastic exudate except just about the cecum where the omentum was bunched up about the gangrenous cecum Cecum was ruptured in pulling away the adherent bowel

Dog XXXIX —(Control) Killed after twenty seven days while in good condition Many dense adhesions about the cecum No fluid or evidence of active peritonitis *Control did much better than the test animal*

*Resume of Series IV* —Only in the last two animals was there a difference In this case the control did much better than the test animal

Recently Dr Little's class has again had the opportunity to try out the same experiments in the Dog Surgery Class The results this year confirm those reported here and repudiate those obtained by the class last year

In the light of these experiments it seems that *bacteriophage* is distinctly contraindicated in the abdomen when the diseased area is not walled off After a wall of plastic exudate has been formed and a definite pyogenic membrane has developed, we still believe that bacteriophage is excellent treatment In that case, or

course, the filtrate does not at all enter the peritoneal cavity and apparently acts in exactly the same way in an abdominal abscess cavity that it acts in other abscess cavities

It has been repeatedly noted that bacteriophage injected into an abscess causes a rapid liquefaction of the exudate thereby facilitating prompt and complete evacuation of the cavity. This action is so marked that occasionally physicians have objected to its use on the grounds that it caused too great a slough. We have felt however, that this criticism has not been well founded because the cavities have healed promptly as a rule and with a minimum of scar production. The mechanism of this liquefaction is suggested when we remember that bacteriophage stimulates the leucocytes as is shown by the increased flow of pus and increased white cell count in the blood.

This negative report is made in the hope that it will deter physicians from using bacteriophage in the abdomen either before or during the operation. We believe that *it should be used only after the diseased area is well walled off*.

#### SUMMARY

- 1 Staphylococcus streptococcus-B coli bacteriophage will not immunize the peritoneum as used in this experiment
- 2 It will not prevent a peritonitis in dogs as a result of tying off the cecum when used at the time of operation
- 3 It seems to inhibit the formation of walling off plastic exudates when used before or during the operation
- 4 Experience other than that mentioned above indicates that it is of value in the treatment of abscesses already walled off whether they are in the abdominal cavity or elsewhere

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### LOCALIZED AREAS OF ISCHEMIA ON THE HANDS\*

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#### HISTORICAL DATA

**B**IER,<sup>1</sup> in 1898, described multiple white spots which occurred on the hand after he had occluded the blood supply in the cubital region by means of a rubber constrictor. Since then, many workers have enlarged upon Bier's original findings, and have also described red spots as being present also. Gilding<sup>2</sup> has produced Bier's spots in animals. Wolf,<sup>3</sup> Boas,<sup>4</sup> Krogh,<sup>5</sup> Lewis,<sup>6</sup> and others have contributed much to our knowledge in respect to the intimate anatomy and physiology which is concerned in the production of these spots.

In 1919, Tracy<sup>7</sup> described chronic noninfectious vasoconstriction spots in man and linked the presence of these spots to organic neural disease. Pigment spots were present at the periphery of the white spotted regions. Also the presence of anemic dermatography was noted as coexisting in some patients with white spots.

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## ANATOMY AND PHYSIOLOGY

Spalteholz<sup>8</sup> claims that the number, and often the size, of the blood vessels to the skin, especially the palms of the hand and the soles of the feet, are greater in number than in other cutaneous areas. There exists an intimate anastomosis between the arteries which produce a cutaneous arterial network (Lewis). The subpapillary arterial network arises from the superficial network and proceeds outwards. Numerous small branches arise from the subpapillary network and thus form the terminal arterioles which supply the superficial layers of the skin and run parallel with it. Spalteholz claims that the cutaneous arterial network has thick muscular coats.

The vasomotor nerves, a part of the autonomic nervous system, enter the muscular coat of the blood vessels by means of their nonmedullated fibers. These vasomotor nerves constrict and dilate these cutaneous vessels. The vasomotor nerves either act by stimulation or depression of the vasomotor centers in the medulla and spinal cord, or they act by means of the humoral mechanism through the blood supply.

## ETIOLOGY

It has been well established that vasoconstriction is responsible for these transient multiform white areas. It is our belief that this phenomenon may be but a further example of the spasmodic action of the blood vessels which is observed in other clinical diseases, such as Buerger's and Raynaud's diseases, erythromelalgia and angina pectoris, as well as in angospasms of the brain, as has been described by Ten Broeck,<sup>9</sup> Osler,<sup>10</sup> and others. The action of the muscular contractions in asthmatics may also point to the above diseases as being of an anaphylactic nature. Angospasms have anoxemia as their end result. These vascular spasms may be caused by the neurotropic action of the media in the blood vessels. Some observers claim that the hydrogen ion concentration is the basic cause. Other workers claim that this angospastic action is the result of sclerotic vascular changes, or hypertension, or even both.

It is our belief that one of the main etiologic factors of the condition of localized ischemia in the hand, is the natural condition of the patient. These people presenting this lesion are of a nervous, high strung type. Contrary to Tracy's belief, there was found no evidence of coexisting brain disease or other neural pathology. There was no vascular spasm in the feet. This may be due to the more marked muscular activity of the hands, more exposure to the elements, and the thinner cutaneous coverings of the hands.

## CASE REPORTS

CASE 1 — A white male student, aged twenty eight, exhibited some spots (white) which he had noticed on both hands. He had these patches as long as he could remember. Physical examination revealed no apparent pathology in any part of his body, except that he exhibited a chronic scaling of his scalp, a pronounced sinus arrhythmia (no correlation with the respiratory phases), and a persistent insomnia. His blood Wassermann was negative. His past and present health was good. None of his immediate family had the white spots on their bodies. Dermatologically, transient white macules were observed on both hands. These spots extended from the region of the wrists to the finger tips. These patches were present on the dorsal and ventral surfaces and were irregular in outline, the average was 4 to 8 mm in diameter. It was observed that these multiple white spots had an average duration of approximately fifteen to forty five seconds and

then disappeared only to reappear elsewhere in the region which we have described. These white macules would disappear when the surface of the hands were either gently rubbed or elevated above the head, and would rapidly reappear when the hands were hung at the patient's side.

CASE 2—A white male, aged twenty eight, whose previous occupation was that of an office worker, presented transient white macules which were continually present as long as he could remember. Physical examination revealed the patient to have a characteristic Parkinsonian syndrome of eleven years' duration. These spots had been noted previous to the onset of encephalitis. His blood Wassermann was negative. He experienced difficulty in walking, which was a part of his Parkinsonian. He had a decided scaling of his scalp, sinus arrhythmia (no correlation with the respiratory phases) and was troubled with insomnia. He states that his father also has the white spots on his hands. These multiple white macules were in the same location as in the other case. They average about 6 to 10 mm in diameter. Their average duration was approximately one minute. They disappeared only to reappear in another location. Rubbing the hands or elevating them above the patient's head caused them to disappear, while lowering the hands to the patient's side caused them to reappear quite rapidly.

#### SUMMARY

1 Two cases of multiform, small areas of transient ischemia, which are continually residual in the patients, are described.

2 These white macules resemble Bier's spots and the macules described by Tracy as "chronic vasoconstrictor spots."

3 There was no evidence of the presence of associated pigment spots, nor was there any associated anemic dermatography present in these patients.

4 It is believed that these spots occur in patients of a neurasthenic constitution. These patients showed a hyperactive nervous system manifested by sinus arrhythmia and insomnia. It is not our belief that these macules are associated with neural pathology, as Tracy has stated.

5 These multiform areas of localized ischemia on the hands are not uncommon, although not described in any of the standard dermatologic textbooks. They may be easily confused with vitiligo.

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## THE NONSPECIFIC FIXABILITY OF GUINEA PIG SERUM WITH BORDET'S ANTIGEN\*

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THE possibility that the guinea pig serum used as complement in the Wassermann test might enter into nonspecific combination with the lipoidal antigens has been taken into consideration by all careful users of this reaction. Special mixtures to supply information on this point are often included as controls in the tests and titrations of complement. The usual controls, however, have not always been adequate, as they have been devised to test for nonspecific fixability of the complement chiefly with the cholesterinized antigen. The record of a recent experience, to be described in this paper, indicates that it is equally important to test for nonspecific fixability of complement with the acetone-insoluble fraction of heart muscle lipoids, known as the Bordet antigen.

The Rochester Health Bureau Laboratories follow very closely the New York State Laboratory Wassermann technic.<sup>1</sup> As a part of that technic, two controls on the anticomplementary properties of each antigen employed are used, 0.1 c c, the same amount that is used in the routine fixation test and four times that amount, or 0.4 c c. These controls are supposed to hemolyze after 15 minutes' incubation in the water-bath at 37° C, to show that the antigen is not anticomplementary. It was occasionally observed that while both controls for the cholesterinized antigen, and usually the 0.4 c c control of the Bordet antigen, cleared promptly, the 0.1 c c amount of Bordet antigen would fail to hemolyze.

It was also observed that occasionally most spinal fluids tested on a certain day, would give reactions with the Bordet antigen, while either none, or only those with a possible luetic history, would give reactions with the cholesterinized antigen. Serums tested on the same day failed to show this unusual result. It was found that these reactions of spinal fluid with the Bordet antigen took place on the days when the controls of the Bordet antigen were apparently anticomplementary.

The Bordet antigen used in these tests was an acetone-insoluble fraction of lipoids of beef heart muscle prepared at the New York State Health Department Laboratories. It is described in detail in "Standard Methods of the Division of Laboratories and Research of the New York State Department of Health" by Dr. Augustus B. Wadsworth in 1927<sup>1</sup> (pp. 182, 189), as follows:

"Remove the fat, blood vessels, pericardium, and endocardium, and cut up the muscle into cubes about one inch in diameter. Then grind the heart muscle in a meat chopper. Weigh the ground tissue, and for each 100 grams add 125 c c of 95 per cent alcohol.

"The purpose of the alcohol is to coagulate the proteins and not to dissolve

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the fatty substances or lipoids, and, as the alcohol is in comparatively small proportion and is immediately diluted with the tissue juice, it exerts but slight dissolving power

"Let the mixture stand at room temperature for several days, then filter, spread the residue on trays in a thin layer and dry in the incubator for a day. To the dried tissue add 200 c c of acetone for each 100 grams of tissue originally used. Mix and leave at room temperature for one week, shaking for a minute or two once a day, then filter. Add 200 c c of fresh acetone for each 100 grams of tissue originally used, mix, and filter the next day. Remove the tissue to trays, and dry in the incubator for one day, then pulverize it finely in a mortar. To the ground tissue add 200 c c of 95 per cent alcohol for each 100 grams of tissue originally used. Mix and allow to extract at room temperature for ten days, shaking for a minute or two three times a day. Then remove the alcoholic extract by filtration through soft filter paper, and store in the dark at room temperature."

"Dilute the acetone-insoluble antigen as follows. Evaporate to dryness one part of antigen, by pipetting the antigen on a watch glass of about six-inch diameter or in an evaporating dish and placing it in front of an electric fan. Then suspend the dried residuum in two and one-half parts of distilled water. For example, if 3 c c of antigen is evaporated to dryness, suspend the dried residuum in 7.5 c c of distilled water. Add the first cubic centimeter slowly with a 0.2 c c pipette and mix as thoroughly as possible with a glass rod after the addition of each 0.2 c c. The remainder of the water may be added rapidly, but make sure that the suspension is thoroughly mixed. Add the appropriate amounts of this suspension to the amounts of 0.85 per cent salt solution necessary to make the dilutions desired. Mix well by shaking."

In this laboratory the antigen is dried in an evaporating dish by means of a small stream of filtered compressed air.

The cholesterinized antigen, which rarely gave fixation tests with guinea pig serums, was an alcohol soluble fraction of lipoids from ether-extracted heart muscle, reinforced with 0.4 per cent cholesterol. It, also, was prepared at the New York State Health Department Laboratories in Albany. The procedure used in the preparation of this antigen is also described in "Standard Methods of the Division of Laboratories and Research of the New York State Department of Health" by Dr. Augustus B. Wadsworth in 1927<sup>1</sup> (pp. 182, 189), as follows:

"Cut and grind the heart muscle, as in preparing tissue for the acetone-insoluble antigen. Then spread it in the thinnest possible layer on a clean, smooth surface, such as a glass plate or stone shelf. Dry before an electric fan for five or six hours, then turn the tissue with a spatula and allow the other side to dry before the fan overnight. Break up the resulting dry sheet of tissue and grind it again. Spread this powder on a tray and dry it in an incubator at 37° C. for three or four days, then pulverize it finely in a mortar.

"Extract the dry, pulverized tissue with successive additions of ether, in the cold room, until the ether is practically water clear, filtering off the ether and replacing it with fresh ether daily. After the final ether extraction, allow the tissue to dry on a tray until the odor of ether is not evident.

"To prepare the alcoholic extract of the dried, ether-extracted tissue, add

10 c c of absolute alcohol per gram of tissue and boil for one hour under a reflux condenser. After cooling to room temperature, filter the extract through soft filter paper. In case a precipitate forms after standing, refilter before adding cholesterol.

"Cholesterinize 100 or 200 c c portions of this extract as needed, by adding 0.4 gram per 100 c c. Weigh the cholesterol accurately on an analytical balance, place in a clean bottle, and add the measured quantity of alcoholic extract. Shake the mixture, and, if necessary, crush large particles of the cholesterol with a glass rod. Stopper the bottle tightly and place in the water-bath at 37° C until the cholesterol is completely dissolved. Leave at room temperature for twenty-four hours and filter, if a precipitate is then evident.

"Dilute the cholesterolized antigen as follows. Pipette the antigen to the bottom of a one liter beaker. Place the required amount of 0.85 per cent salt solution in another liter beaker. Pour the salt solution onto the antigen as rapidly as possible and mix thoroughly by pouring back and forth from one beaker to the other, several times." (In this laboratory 100 c c bottles instead of one liter flasks are used.)

"Antigen, before use in complement fixation tests, is titrated for lytic, anti-complementary, and antigenic properties, the anticomplementary and antigenic properties being determined from titrations with at least twenty different specimens of pooled complement, during a period of at least four weeks. The dilution of optimum sensitivity, within the range which is antigenic but not lytic or anti-complementary, is then determined by titrations and tests of syphilitic sera."

Antigenic dilutions were never used under fifteen minutes, or over two hours, old in this laboratory.

The question arose as to whether this inhibition of hemolysis was due to an anticomplementary action of the antigen itself, or to the complement. In order to determine whether the inhibitory effect was due to the antigen, the following procedures were carried out: (1) Varying the length of time of drying the antigen before dilution, (2) Drying the antigen by compressed air, or by means of the electric fan, (3) Adding the total amount of distilled water in diluting, in 0.2 c c amounts, or by following the directions on the bottle, and adding only the first 1 c c in 0.2 c c amounts, (4) Prolonging emulsification in the distilled water, or fairly rapid emulsification, still following the directions on the bottle, (5) Using the stock normal salt solution, or using a special normal salt solution made by weighing the salt on the analytical balance, (6) Considering the possibility that the antigen dilution might be hemolytic for sheep cells, and that in the 0.4 c c antigen control, this hemolytic property might veil the anticomplementary property. To test this, 1-10, 1-20, 1-30, and 1-40 as well as the routine 1-50 dilution were made up, and 0.1 c c and 0.4 c c of each dilution were tested with 5 per cent cells. No hemolysis occurred even in the 1-10 dilution.

Varying these different conditions in the preparation of the antigen made no difference in the final result. This led to the conclusion that the factors producing the result must be in the complement.

It was decided, therefore, to test the individual guinea pig serums for non-specific fixability with Bordet's antigen, in addition to the test which had always

been carried out routinely with the cholesterinized antigen, in conformance with the New York State Laboratory Wassermann technic. The test was performed according to the following directions: Prepare a 1-10 dilution of each guinea pig serum, use 0.1 c.c. serum dilution, add 0.2 c.c. antigen dilution, fix for two hours in the refrigerator at from 3° to 6° C, remove from the refrigerator and add 0.2 c.c. sensitized cells, incubate in the water-bath at 37° C until hemolysis is complete, record the time required for complete hemolysis with each guinea pig serum. Include in the pooled complement only the serums with which the sensitized cells are hemolyzed in less than ten minutes, the others show nonspecific fixability and are discarded as unsuitable for use.

In the course of these experiments, serums giving nonspecific fixability were occasionally included in the pool of complement for the next day's work, sometimes because the majority of serums tested were anticomplementary and had to be used, and sometimes to observe the effect in the pooled complement and on the antigen controls. After the tests were completed, the 1-10 dilutions were inactivated, and complement fixation tests carried out on them on the following day. Each day a sample of the pooled diluted complement was also inactivated, and a complement fixation test done on it.

Between April 27, 1931, and October 3, 1931, 400 guinea pig serums were tested. Of this number, 106 or one-quarter, showed nonspecific fixability with the Bordet antigen, but only 40 with the cholesterinized antigen. Approximately one-half (198) gave reactions in the Wassermann test with the Bordet antigen, but only 34 with the cholesterinized antigen. There were only 5 serums which showed nonspecific fixability with the Bordet antigen, which did not give reactions in the Wassermann test, but there were 98 serums which did not show nonspecific fixability in the preliminary test, which did give reactions in the Wassermann test.

The suggestion is made that the fact that the tests for nonspecific fixability are held for two hours at ice box temperature, while the complement fixation tests are held for four hours, may be of influence here. A 1-20 dilution of guinea pig serum for the test for nonspecific fixability is advised by the State Laboratory, and used there, and it is possible that the anticomplementary quality of some of the guinea pig serums was masked by the stronger hemolytic activity of the 1-10 dilution.

Kahn tests were done on 60 of the 106 serums which gave nonspecific fixability with Bordet's antigen. All Kahn tests were negative.

An examination of the records was made, to see whether the length of time required for hemolysis at 37° C in the test for nonspecific fixability bore any relation to the positive Wassermann tests. Following are the figures for this time required for hemolysis, ten minutes, 1 serum, nine minutes, 3 serums, eight minutes, 8 serums, seven minutes, 15 serums, six minutes, 17 serums, five minutes, 9 serums, four minutes, 19 serums, three minutes, 26 serums. There were as many serums that hemolyzed rapidly, giving reactions in the Wassermann test as serums that hemolyzed slowly.

The age, when tested, of the 1-10 dilution of guinea pig serums had no bearing on the positive results in the Wassermann tests. Sixty-five of the 98 serum dilu-

tions were twenty-four hours old when tested, while only 33 were forty-eight hours old

These results show that neither the length of time required for complete hemolysis in the preliminary test, nor the age of the 1-10 serum dilutions when tested, were responsible for the positive reactions in the Wassermann test

Records were kept of the 112 pooled diluted complements which were used during the period while the 400 serums were being tested. Of these 112 pools, 103 had samples inactivated, and Wassermann tests done. A table has been made showing the relation between the presence of a reaction with the Bordet antigen in the pooled complement and whether or not the Bordet antigen controls were anticomplementary

Wassermann -	antigen controls negative	47	} 85.44% agreement
Wassermann +	antigen controls anticomplementary	41	
Wassermann -	antigen controls anticomplementary	15	
Wassermann +	antigen controls negative	0	
Total		103	

Of the 15 pooled complements having negative Wassermann tests and the Bordet antigen controls anticomplementary, 13 pools had included in them serums which gave reactions in the Wassermann test. Of the 56 pooled complements which had 0.1 c.c. Bordet antigen controls anticomplementary, only 21 had the 0.4 c.c. controls anticomplementary, also

The 400 serums, the results on which have been presented, do not represent 400 guinea pigs, but instead, 400 consecutive bleedings. To determine the incidence of this type of reaction in guinea pigs used here, serums from 100 consecutive guinea pigs were tested. There were no duplicates in this series. Of the 100 guinea pig serums, 35 either showed nonspecific fixability with Bordet's antigen, or gave a reaction with Bordet's antigen in the complement fixation test. Sixteen of the 35 guinea pigs were males, and 19 were females.

There were 100 guinea pigs which had two or more bleedings. Of these, in 46, the serum was always satisfactory, in 17, it was always unsatisfactory with Bordet's antigen, and in 37, the results of the bleedings varied. Of the satisfactory 46, 21 were males and 25 were females, of the 17 unsatisfactory, 9 were males and 8 were females, of the 37 variables, 22 were males and 15 were females. These tests were carried on into January, 1932, in order to include in this paper the results on as many guinea pigs as possible.

Apparently the sex of the guinea pig is not responsible for the nonspecific fixability with Bordet's antigen, nor for its variability in the same guinea pig.

Twelve of these guinea pigs were examined for lesions suggestive of spirochetal infection of the genitalia. No pathologic conditions were seen. Secretions from the genitalia of these animals were examined by dark-field illumination. Coarse, thick spirochetes and smaller short spiral forms were found in specimens from 7 of the animals. None of the spirochetes resembled *Spirocheta cuniculi*.

A search of the literature revealed an article by V. Tomasek on this subject.<sup>2</sup> The author noted that some individual samples of guinea pig serum reacted nonspecifically with Bordet's antigen, but gave no reaction with Kolmer's antigen

He found that cold weather, and lack of green food in winter, were contributing factors because the largest number of unstable complements were found at this time. But he thought the method of preparation of the antigen was the cause, and that it was probably due to the *preliminary* extraction of the beet heart with acetone, since plain alcoholic extracts, and the preliminary extraction with ether (as in Kolmer's antigen) do not result in nonspecific fixability. He thought it probable that the extraction with acetone changed the proportion of the lipoids of the extract, and that this quantitative change might be the cause of the anti-complementary power.

Tomasek thought it extremely important to test each guinea pig serum for anticomplementary properties, and not to pool such serums with those which reacted normally, because the mixture of poor and normal complement is sometimes fixed by Bordet's antigen in the same way that the weak complement itself is.

The tests carried out here were during late spring, summer and early fall, when cold weather was not a contributing factor.

This paper was presented before the meeting of the New York State Association of Public Health Laboratories in Albany on November 8, 1931. Discussion brought out the fact that at the State Laboratory where preliminary tests for nonspecific fixability with both antigens are done on all guinea pig serums to be used as complement, only a small number of serums react with Bordet's antigen. This does not confirm our results. Another laboratory in the state, which carries out a large number of Wassermann tests, but which does no preliminary tests on guinea pig serums, reported that it also found many spinal fluids positive with Bordet's antigen, but not with the cholesterinized antigen, this bears out our findings.

It was suggested that possibly the diet of the animals might be an important factor. The guinea pigs used here are fed, the year round, on a diet of cabbage, alfalfa, and oats. The alfalfa is cut from the grounds surrounding the animal house and in the summer it is fed to the animals green, but in the winter it is dried. Ninety per cent of the guinea pigs used here are purchased from dealers in Rochester and vicinity.

It was also suggested that the reason why serums were not affected like spinal fluids in the Wassermann tests was due to the buffer action of the serums.

No suggestion was offered as to why the 0.4 cc antigen controls often hemolyzed while the 0.1 cc controls did not.

Since this paper was presented, preliminary tests for nonspecific fixability with both antigens were carried out, using both 1-10 and 1-20 serum dilutions. One hundred and ninety consecutive serums were tested. With Bordet's antigen, 23 serums showed nonspecific fixability with the 1-20 dilution, and 19 with the 1-10 dilution. With the cholesterinized antigen, 25 showed nonspecific fixability with the 1-20 dilution, but only 11 with the 1-10 dilution. These figures show that the 1-20 dilution shows up many more cases of nonspecific fixability with the cholesterinized antigen than does the 1-10 dilution, but that with Bordet's antigen, the difference between the two dilutions is much less marked. In the 1-20 dilution about the same number of guinea pig serums reacted with each antigen, about one-half (13) reacted with both antigens.



It seems evident that it is better to use a 1-20, than a 1-10 dilution of guinea pig serum for tests for nonspecific fixability

#### SUMMARY

The results presented in this paper show

1 Guinea pig serum is more apt to show nonspecific fixability with Bordet's antigen than with the cholesterinized antigen (106 out of 400, as against 40)

2 Serums showing nonspecific fixability with Bordet's antigen will usually give reactions in the Wassermann test (only 5 out of 106 did not)

3 Many serums in a 1-10 dilution not showing nonspecific fixability with Bordet's antigen will give a reaction with the Wassermann test (98 out of 400 did) Some of these might have shown nonspecific fixability if tested in a 1-20 dilution

4 These serums showing nonspecific fixability with Bordet's antigen give negative results with the Kahn test (All 60 tested out of the 106 were negative)

5 The length of time required for hemolysis in the test for nonspecific fixability, and the age of the 1-10 dilutions of guinea pig serum when tested, have no bearing on the occurrence of a reaction in the Wassermann test

6 There was fairly good agreement (85.44 per cent) between the presence or absence of a reaction in the Wassermann test on the pooled diluted complement, and the presence or absence of anticomplementary action in the Bordet antigen controls

7 The serums of 35 per cent of 100 different pigs bled and tested consecutively showed nonspecific fixability with Bordet's antigen, or gave reactions with Bordet's antigen in the complement fixation test, or both

8 Of 100 guinea pigs which were bled two or more times, in 46 the serum was always negative with Bordet's antigen, in 17 it was always unsatisfactory, and in 37 variable

9 The sex of the guinea pig did not seem to be a factor in the quality of nonspecific fixability with Bordet's antigen, or of its variability in the same guinea pig

#### CONCLUSIONS

Since at least one-third of the guinea pig serums tested in this laboratory were not suitable for use with Bordet's antigen, and since the inclusion of unsuitable serums in the pooled complement gave rise to apparently nonspecific reactions with Bordet's antigen on spinal fluids, it is obvious that all guinea pig serums should be subjected to a preliminary test for nonspecific fixability with Bordet's antigen, and only those serums which do not react with this antigen should be included in the pooled complement

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## THE SPECIFICITY OF THE KAHN TEST IN MALARIA\*

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THERE have been many reports in the literature of nonspecific Wassermann reactions in malaria. Weil and Braun<sup>1</sup> report false positives in malaria. Others using a wide variety of technique report false positive reactions, but Kolmer<sup>2</sup> states that "The sera in acute febrile diseases may become somewhat more anticomplementary than usual but with due care in technique these nonspecific positive Wassermann reactions can be avoided." The fact of reported false positive Wassermann reactions in malaria has perhaps led to an idea that positive serology in malaria patients is not trustworthy.

I am unable to find reports of false positive Kahn reactions in malaria, but, in regard to the febrile diseases, Jun-Wu-Mu and Keim<sup>3</sup> report a series of Kahn and Kolmer examinations in febrile diseases including pneumonia, typhus, relapsing and typhoid fever. They find that the Kahn reaction is not appreciably affected by the febrile reaction in these diseases. Besancon and Mayer<sup>4</sup> made a study of the Kahn reaction during chill and fever due to nonspecific protein. They state that the Kahn test shows a tendency toward negativity in both chill and fever.

The blood examinations reported in this communication were done in the hospital of the Cia, Foid Industriel do Brazil, situated in the Amazon River Valley, four degrees south of the equator. This is a highly malarious section and one in which the incidence of syphilis is known to be high. Yaws is also fairly prevalent. Maxwell<sup>5</sup> and others have shown that positive Kahn reactions are obtained from the blood of patients suffering from yaws. Badger<sup>6</sup> has shown that the incidence of positive Kahn tests in leprosy is high, and, since leprosy is very prevalent in the Amazon valley, this factor cannot be discounted. However, none of the patients in this series were suffering from either leprosy or yaws in a stage that could be recognized clinically.

The routine diagnostic test with serum as described by Kahn was done in these cases. The antigen was purchased from American biologic houses manufacturing it.<sup>7</sup> Readings were made in accordance with the instructions given by Kahn.<sup>8</sup>

The procedure was as follows:

Blood smears for malaria and blood for Kahn tests were obtained routinely from newly admitted patients. The malaria smears were stained with Wright's stain and examined immediately. The Kahn tests were done at intervals of from two to three days. The temperature recorded here is the admission temperature. Repeat examinations were done on those patients who could be located after three to six weeks.

The results of the tests fall into the following groups:

Positive malaria and negative Kahn	116
Positive malaria and positive ++++ Kahn	61
Positive malaria and positive +++ Kahn	6
Positive malaria and positive ++ Kahn	11
Total	<u>194</u>

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The above figures show 60 per cent of the Kahn reactions negative, or that a preponderance of patients acutely sick of malaria have negative Kahn reactions

On the other hand 40 per cent of the Kahn reactions are positive which is certainly a high percentage. However, the incidence of clinical syphilis is very high, numerous cases of yaws are seen and leprosy is widespread. The general incidence of positive Kahn reactions is 41 per cent, 452 positive in 1099 reactions. So that the incidence in this group of malaria patients is slightly less than the general incidence.

Of the positive Kahn reactions it was possible to repeat 45 after an interval varying from three to six weeks. At this time all were clinically well of malaria, without fever, and showed negative blood slides.

*Results of repeat examinations (positive Kahns) —*

Reaction same as first test	39
Increased from ++ to +++	3
Increased from +++ to ++++	1
Decreased from +++ to ++	2

The group of 4 patients whose reaction had increased had taken no treatment, while those 2 whose reaction had decreased had each taken 6 injections of neosalvarsan.

Eleven of those who had negative Kahn reactions and positive blood smears were repeated when free of malaria. Their Kahn tests all remained negative.

*Relation of admission temperature to Kahn reactions —*

Of the 78 patients who showed both positive Kahn reactions and malaria on examination, the admission temperature was as follows:

Normal	37° C	13
Subnormal	35°-37° C	18
Abnormal	37°-40° C	47

Of the 116 patients showing negative Kahn test and positive malaria, the admission temperature was as follows:

Normal	37° C	16
Subnormal	35°-37° C	31
Abnormal	37°-40° C	69

These two tables show that essentially the same temperature reaction occurs in the group showing positive Kahn tests as in the group showing negative Kahn tests, 60 per cent of those with positive Kahn tests show fever, while 59 per cent of those with negative Kahn tests show fever. These tables also show that the febrile group of 116 patients yield 40 per cent positive Kahn reactions, and in the group showing no fever the incidence of positive Kahn reactions was also 40 per cent.

#### SUMMARY

Kahn tests were done on the blood of 194 patients who were acutely sick of malaria.

The incidence of positive Kahn tests is slightly less in this group than it is in all admissions to this hospital.

Repeat examinations of 56 patients were done and yielded essentially the same result as the first examination

The preponderance of Kahn tests are negative

Those having fever show the same incidence of positive Kahns as those not having fever Both of these figures are approximately the same as the general incidence of positive Kahn tests

#### CONCLUSIONS

The specificity of the Kahn test is not affected by acute malaria, or its febrile reaction

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HENRY FORD HOSPITAL

# LABORATORY METHODS

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## A COLORIMETRIC METHOD FOR THE ESTIMATION OF UROBILINOGEN IN URINE AND FECES

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THE reaction of Ehrlich<sup>1</sup> for the recognition of urobilinogen in urine is too well known to need but a casual reference since it was first described by him in 1901. While investigating the aniline dyes for their effects upon trypanosomes, Ehrlich noticed that the addition of paradimethylaminobenzaldehyde to certain fresh urines produced a bright red coloration.

Two years later Pappenheim<sup>2</sup> called attention to the fact that the reaction only occurred in those urines that, on standing, gave the reaction for urobilin. Later, Neubauer<sup>3</sup> demonstrated that the reaction was due to urobilinogen, a colorless chromogen which gradually becomes converted into urobilin by the action of light and oxygen.

Although after these many years rather strong criticisms of the reaction have appeared, the simplicity of the test has always insured for it a permanent, if not paramount place.

It was therefore decided to ascertain how far the reaction could be applied for a quantitative method. Investigation showed that it was capable of application to urine and feces, and that this quantitative process preserves the simplicity of the original reaction.

The limitations of this process arose from the fact that if the red coloration produced by the aldehyde salt from urobilinogen were to be compared against a standard solution (and it follows that for convenience the standard must be an artificial one), the yellow color of the urine would seriously interfere with the comparison. Also the yellow color of the reagent itself, as well as the yellowish green color produced by it from urea, would render comparison impossible. Then there are the other various practical points of difficulty that have been raised in the past which have to be considered. They may be divided into the following categories:

1. The reaction is not consistent with results, due presumably to the many variations in the technique recommended to bring about the reaction. This point alone emphasizes the need for a standard method.

2. Likewise, the ingestion of certain drugs such as hexamethylamine or antipyrine are known to produce urobilinogenuria.

3. The presence of acetone in the urine must also be excluded as it produces a similar coloration.

4. And again the effect of hydrochloric acid in excess on other urinary pigments produces a marked red coloration; hence an excess of the aldehyde salt must be avoided.

First, the problem resolved itself into finding a quantitative method employing the aldehyde salt of Ehrlich, and that the red color so produced from urobilinogen

under standard conditions, to be freed from the yellow color of the urine, and then compared against a standard solution

Second, the presence of drugs in the urine do not cause a reaction characteristic of the protochrome, but, of course, the urobilinogenuria caused by the ingestion of drugs is not preventable

Third, this special dilution employed prevents acetone and diacetic acid reacting to give the slightest red coloration

Fourth, the amount of hydrochloric acid introduced with the benzaldehyde salt is such that no reaction can occur with the urinary pigments

The method as presented here may be summarized as follows

A measured amount of urine is treated with an appropriate amount of a specially prepared solution of paradimethylaminobenzaldehyde. After standing for a given time the mixture is heated under standard conditions in a boiling water bath for a short period, and after cooling the coloration so produced is extracted with amyl or butyl alcohol. This extracted red coloration which is practically free from foreign material and other interfering colors, is then compared in the colorimeter against a standard ferric thiocyanate solution almost identical with that employed by Van den Bergh<sup>4</sup> for the estimation of bilirubin in blood

The urines examined are from such cases where urobilinogen appears in the urine, i. e., with hepatic diseases, diabetes mellitus, tuberculosis, infectious diseases, visceral hemorrhage, and heart diseases in stage of decompensation

#### Solutions

1. A special dilution of Ehrlich's benzaldehyde salt. Paradimethylaminobenzaldehyde 10 gm. Pure concentrated HCl 100 c.c. Distilled water to 300 c.c. To about 100 c.c. of distilled water add the 100 c.c. of concentrated hydrochloric acid, mix and then add 10 gm. of paradimethylaminobenzaldehyde. Finally make the volume to 300 c.c. with distilled water

2. A saturated aqueous solution of ammonium sulphate

3. Pure amyl and butyl alcohol

4. Special large thick walled test tubes (Monax combustion tubes 6 inches by  $\frac{5}{8}$  inch) graduated to hold about 25 c.c.

5. Standard ferric thiocyanate solution as modified by the author in a recent paper

**Stock Solution A**—Dissolve 0.1508 gm. of ammonium iron alum in 50 c.c. of concentrated hydrochloric acid, and then add water to 100 c.c.

**For Stock Solution B**—Take 10 c.c. of Solution A, plus 25 c.c. of concentrated hydrochloric acid, and water to 250 c.c. This final solution is quite stable and keeps for several months. Then for the comparison standard take 3 c.c. of Solution B, plus 3 c.c. of 20 per cent potassium thiocyanate, mix well, and extract with 12 c.c. of amyl or butyl alcohol depending upon the alcohol used for the extraction of the unknown solution

This standard corresponds to a concentration of 1 in 200,000 of azo-bilirubin, or 0.5 mg. (1 Van den Bergh unit)

As there seems no objection to this arbitrary standard both for quickness and simplicity it has been adopted for the aldehyde reaction, substituting urobilinogen for the above dilution of 1 in 200,000 of azo-bilirubin

#### METHOD

To 10 c.c. of a fresh urine in one of the thick walled test tubes add 0.5 c.c. of Ehrlich's reagent. After standing for ten minutes at room temperature to allow

"coupling" to take place, immerse the tube in a beaker of boiling water for exactly one minute. Then cool immediately in running water. The coloration so produced is extracted by adding 1 c.c. of saturated solution of ammonium sulphate plus 10 c.c. of amyl or butyl alcohol, and shaking thoroughly for about one minute. Now allow the alcohol roughly to separate, after which it can be pipetted or decanted into a large centrifuge tube and then spun for a few minutes to separate the alcohol completely.

It would be as well to mention before proceeding that experience has shown the butyl alcohol to be superior to the amyl alcohol, in that it does not produce such a persistent emulsion with protein and such like interfering substances as does the amyl alcohol. It can also absorb a far greater amount of "azo-urobilinogen" which is to a distinct advantage when used for the feces process where strong colors have to be dealt with. The extracted reddish pink color produced from the urine is then compared against the non rhodanate standard which should be of half strength (15 c.c. Solution B, 15 c.c. of 20 per cent potassium thiocyanate, 12 c.c. butyl alcohol), as this is most suitable for comparison with the majority of urines. A correction must, of course, be made in the final calculation to account for this.

As regards the unknown solution a characteristic reddish pink color is only obtained in those urines where the urobilinogen content is raised above the so called "normal" limits, while with strictly normal urines the alcoholic extract is colored a faint brown tinge, which is quite incomparable against the ferric thiocyanate standard.

The calculation is as follows

$$\frac{\text{Standard}}{\text{Unknown}} \times \frac{\text{Alcoholic extract in c.c. (10 c.c.)}}{\text{Amount of urine used (10 c.c.)}} \times \frac{\text{Strength of standard}}{(0.5 \text{ or } 0.25 \text{ mg., i.e. } 1 \text{ or } 0.5 \text{ unit})}$$

equals units of urobilinogen per 100 c.c. of urine

Table I shows some of the findings by this method

TABLE I

TYPE OF CASE	UROBILINOGEN	
	IN UNITS	IN MG.
Thirty normal subjects	0 to 0.50	—
Three cases of diabetes mellitus	{ 0.33 0.44 0.89	0.16 0.22 0.44
Heart disease	28.00	14.00
Two cases of pulmonary tuberculosis	{ 0.23 0.59	0.11 0.29
Pernicious anemia	2.50	1.25
Postoperative cholecystectomy	4.00	2.00
(A) Acholuric jaundice	2.53	1.26
Typhoid fever	3.55	1.77
First stages of catarrhal jaundice	7.85	3.92
(B) Hemolytic jaundice	5.20	2.60
(C) Cirrhosis of liver	10.55	5.27
(D) Anemia	1.85	0.92
(E) Splenic anemia	2.49	1.24
Pulmonary tuberculosis	1.61	0.80
Hemolytic jaundice	14.30	7.15

From the above results it will be observed that a reaction is only obtained if the urobilinogen content is over 0.20 unit and if below this amount, as previously stated, only a brownish-yellow tinge is extracted, and that the real abnormal urobilinogen content of the urine is above 0.50 unit, but that urines from apparently normal individuals may give a reaction which lies between 0.20 and 0.50 unit.

Before proceeding with the details of the analysis for the urobilinogen content of feces, it will be as well to refer to the slight difference in technique as that used for the urinary process.

First, a suspension of feces is made in water, and after thorough mixing to ensure an even emulsion, the aldehyde salt is added. The amount of this salt added is in excess of that used for the urine analysis, as some of the HCl in the reagent will be buffered by the fecal material. After the standard heating conditions, ammonium sulphate is added to the cooled solution, the addition of which not only aids in the separation of the solid material from the alcohol, but also causes complete extraction of the red coloration, by the butyl alcohol. After centrifuging, the clear red alcoholic layer is transferred to the colorimeter, and compared against the non-rhodanate standard.

Second, as the reddish pink color so produced is most pronounced with the majority of feces it is usual to make the comparison against a double or even a quadrupled standard.

Third, to prevent any pink coloration due to a reaction between indol in the feces and the benzaldehyde solution, the same dilutions are employed as those used for the urine method.

#### METHOD

Into one of the thick walled test tubes weigh out 0.5 gm. of wet feces. Suspend this in about 5 c.c. of water by inserting a rubber stopper and shaking violently to break up any hard solid material that may be present. To the fecal solution is now added 3 c.c. of Ehrlich's reagent and after diluting the volume to 10 c.c. with water, again mix well. After standing at room temperature for ten minutes, immerse the tube in a beaker of boiling water for one minute, and then cool. Two cubic centimeters of saturated solution of ammonium sulphate are added followed by 10 c.c. of butyl alcohol. Now mix thoroughly, and the alcohol is then allowed to separate roughly as specified for the urinary process. Pipette or decant the supernatant material into a centrifuge tube and then spin for five minutes or so, or until the alcoholic extract is clear. If the color of the unknown solution is still too deep even for the quadrupled standard, it may be diluted with a further addition of butyl alcohol, but for most cases it will be found that the double standard (6 c.c. of Solution B, 6 c.c. of 20 per cent potassium thiocyanate, and 12 c.c. of butyl alcohol) is most convenient.

Finally it must be remembered that for the urine or the feces process the operator must himself judge the most suitable standard for comparison with the unknown solution, and also that this comparison must always be calculated as for the 1 unit standard (0.5 mg.)

A small quantity of wet feces, about one gram, is then dried in a small evaporation basin to constant weight, and the necessary correction is made in the final calculation to allow for the water percentage in the feces examined.



The calculation is as follows

$$\frac{\text{Standard}}{\text{Unknown}} \times \frac{\text{Dilution of (10 c c) color in c c}}{\text{Amount of wet feces taken (0.5 gm)}} \times \frac{\text{Strength of standard}}{(1, 2, 3, 4 \text{ units, } 1 \text{ c } 0.5, 1, 1.5, 2 \text{ mg})}$$

equals units of urobilinogen per 100 gm of wet feces

The following form contains the directions for the correction of the water percentage of the feces

Exactly one gram of wet feces is weighed out into a small porcelain dish or crucible and dried to constant weight by means of a boiling water bath and a vacuum desiccator. Suppose for example the weight of the dish plus one gram of wet feces is 18.8060 gm, the weight of the dish alone being 17.8060 gm. After drying, the weight is 18.1936 gm. Therefore, the weight of dry material in 1 gm of wet feces amounts to 0.3876 gm. This figure multiplied by 100 gives the percentage of solid contained in the sample of wet feces. In the example quoted it will be 38.76 per cent.

As the original equation expresses the urobilinogen content in units per 100 gm of wet feces, the result obtained multiplied by

$$\frac{100}{\text{amt of solid material expressed in percentage}}$$

will correct the urobilinogen content for 100 gm of dried feces

The above illustration, as well as the final equation, are selected from Case 1 in Table II given below. The wet feces contained 92.3 units urobilinogen per 100 gm. The solid material was 38.76 per cent. Therefore, 100 gm of dry feces will contain

$$\frac{100}{92.3 \times 38.76} = 238.1 \text{ units}$$

Table II epitomises the results obtained by this method of analysis

Before summarizing the above results, it would be interesting to give some notes of the individual cases examined.

In the urinary analysis of Case A, no bile pigments or salts could be detected, but the Van den Bergh reaction in the blood gave a delayed direct reaction—the indirect test showing 5.5 units of bilirubin.

CASE B—Bile absent in the urine, but the reaction for bile salts was strongly positive. The blood gave a delayed direct reaction with a content of 3.3 units of bile.

CASE C—No bile pigments or salts present in urine. The direct reactions for bile in the blood were negative, but the indirect reaction gave 0.81 units of bilirubin.

CASE D—The urine gave a practically normal content of urobilinogen, but the feces contained a large amount of the chromogen which would be expected in this type of case.

In Case E, the results of the urine, blood, and feces examinations are most interesting. The urine contained 15 units of bilirubin, while the bile salt content was also large. The urobilinogen reaction was of course, negative, as would be expected. The diastase content was 60 units. The blood gave a strong direct reaction for bile, the indirect reaction content being 21 units. The feces gave a completely negative reaction for urobilinogen, proving that the chromogen is decreased or entirely absent in cases of obstructive jaundice.

CASE F—Urine contained no bile pigments or salts. The blood gave negative direct reaction for bilirubin, but a positive indirect reaction corresponding

TABLE II

TYPE OF CASE	COLOR AND NATURE OF FECES	UROBILINOGEN CONTENT		
		UNITS	CORRECTED FOR WATER PERCENTAGE	
			UNITS	MG
Normal feces 1 2 3 4 5 6	In all six cases color normal, light brown			
	Percentage of solid material 38.76%	92.3	238.1	119.0
	23.57%	82.2	348.6	174.3
	30.01%	61.7	205.5	102.7
	34.60%	97.7	282.3	141.1
	21.73%	74.8	344.2	172.1
	42.47%	130.0	306.0	153.0
Pancreatic insufficiency	Very pale yellow Fat content 57.51% Solid material present in 28.01%	27.3	97.4	48.7
(D) Anemia	Dark brown 28.98% solid material	120.0	414.0	207.0
Hemolytic jaundice	Dark dry feces Solid material 31.84%	166.6	532.2	261.6
Pernicious anemia	Dark brown Solid material 32.30%	171.1	529.7	264.8
Cholangitis	Color, brown Solid matter 24.8%	103.0	415.3	207.6
Gross diarrhea	Solid matter 8.84%	67.5	763.5	381.7
Gastroenterostomy	Very pale yellow Gave very strong mercuric chloride (Schmidt's) reaction for bile pigments Unaltered bilirubin present to extent of 133.2 units on V den Bergh scale Solid material 18.01%	50.0	277.6	138.8
(E) Carcinoma of head of pancreas (causing obstructive jaundice)	Very light grey Solid matter, 44.057% Fat content, 41.73%		Urobilinogen completely negative	
Anemia	Dark brown Solid material 19.26%	87.0	452.0	226.0
(F) Splenic anemia	Dark brown feces Solid percentage 21.17%	141.3	667.4	333.7
Catarrhal jaundice	Very pale yellow Solid percentage 52.35%	87.0	166.2	83.1
Five cases of obstructive jaundice	Pale grey		Completely negative	

to 3.2 units. The result of the feces examination for urobilinogen is recorded in the latter tabulation.

Lastly, the results from the feces process may be briefly concluded as follows:

The urobilinogen content of normal feces is much too variable to obtain any normal limits, but it will be observed from Table II that it is much lower than that obtained from such cases as anemia, pernicious anemia, and hemolytic jaundice, where the urobilinogen content is high.

Again with many diseases of the liver such as partial obstruction of the bile passages or complete obstruction, the chromogen content is certainly decreased in the former, and completely absent or mere traces present in the latter disease

In conclusion, just two points of practical importance may be mentioned, these being that the urine and feces samples should be quite fresh and examined as soon as possible, otherwise paradoxical results will be obtained. Also the reddish pink coloration produced from urobilinogen should, if possible, be compared in the colorimeter at once, as fading may occur

#### SUMMARY

1 Use is made of a simple and ready reaction for the detection of urobilinogen in urine. This reaction of Ehrlich is extremely delicate, while the reddish pink coloration produced from the chromogen in urine and feces with paradimethylaminobenzaldehyde, can be matched against a standard solution thus enabling an estimation to be made

2 The quantitative process may enable a positive diagnosis to be made as to whether the amount of urobilinogen is abnormal or not. For instance, in such cases as pernicious anemia or hemolytic jaundice, there is a marked secretion of urobilinogen in the urine and a raised amount in the feces, whereas in cases of partial or complete obstruction of the liver, the chromogen content of the feces is decreased or entirely absent

3 The test is of great practical value for the detection of "latent" jaundice when there is a marked secretion of urobilinogen. In this respect it is more delicate than the Van den Bergh reaction

4 Various alterations in the original reaction have improved the technic and enabled an estimation of the chromogen to be made

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# SURGICAL MAGGOTS IN THE TREATMENT OF INFECTED WOUNDS, CULTURE OF STERILE MAGGOTS\*

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ATTENTION has been directed in the last three years to the beneficial effects produced by blowfly larvae in the treatment of chronic osteomyelitis and other infected wounds. Since the publication in 1929 and 1930 of Dr Wm S Baer's remarkable results,<sup>1,2</sup> from the use of maggots in osteomyelitis, his method has been employed with success in a number of hospitals in the United States and Canada. There have also recently appeared in the medical literature several articles (listed at the end of this paper) dealing with various aspects of culture and treatment and describing results obtained.

This treatment necessitates the production of large numbers of sterile maggots throughout the year, and presents a problem requiring detailed knowledge of a special nature. It is possible for hospitals to purchase sterile maggots ready for use, but often it is more satisfactory to rear them as needed. The present contribution describes in detail cultural methods which are being used with good results in this laboratory, and also discusses the necessary sterilizing technique. The green-bottle blowfly (*Lucilia sericata* Meigen), described in this article, is a satisfactory species for cultural purposes and for use in wounds.

## ESSENTIAL FEATURES OF LIFE HISTORY AND HABITS OF THE BLOWFLY

*The Egg*—Eggs are minute, white, and elongate, and are laid in clusters upon meat. They are soft and easily crushed, and should be handled carefully. Hatching occurs at incubator temperatures within twenty-four hours.

*The Larva or Maggot*—The maggots which emerge from hatched eggs require access to food, otherwise they would perish. For ordinary rearing purposes, therefore, it is best to allow the eggs to remain upon the meat where the flies have laid them. This meat keeps the eggs moist and serves as food for the maggots. For the production of sterile maggots a special food is prepared and will be described later.

The maggot is pointed at the head end and has no eyes. It feeds upon meat and takes up its food chiefly in liquid form. Breathing takes place mainly through two spiracles in the posterior end. Maggots develop quickly and in five to seven days become full grown. They then cease feeding and wander for a time in search of a secluded place in which to pupate. A layer of sand provides a suitable environment for pupation.

*The Pupa*—After it is quiescent the maggot becomes oval in shape and brownish in color. Transformation from the maggot to the fly then takes place and occupies usually about seven days. There is no feeding in the pupal stage.

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In the prepupal stage further development may be delayed for several weeks, if desired, by placing the insects in cold storage at a temperature of 45° to 70° C (40° to 45° F). Normal development may be induced by replacing them in a temperature of 26.5° C (80° F).

*The Adult or Fly*—After the adult emerges from the pupal case it soon commences to feed. Flies have been found by Baer<sup>3</sup> to thrive well upon a mixture of 70 cc of water and 30 cc of honey, to which is added 3 gm (about one-quarter cake) of yeast. A pad of absorbent cotton about 2 inches square is placed in a Petri dish and thoroughly moistened with the mixture. Granulated sugar, as used by Miller,<sup>13</sup> also appears to give good results. With sugar, a water-fountain dish, as described by Murdoch and Smart<sup>14</sup> or Miller should be added. In another dish is placed a piece of lean beef about 2 inches square for the flies to lay eggs upon.

Females commence to lay eggs about five days after emergence. The number laid daily by one fly varies considerably, but averages about 100. The egg-laying period may extend over three to five weeks, but a colony should be destroyed when egg production falls below a satisfactory level.

*Temperature and Humidity Requirements*—All stages from the egg to adult appear to be adaptable to a fairly wide range of temperature—from 24° C (75° F) to as high as 35° C (95° F) in summer. It is best, however, to use a constant temperature in order to control the rate of growth and activity. A temperature of 26.5° C (80° F) has been found satisfactory. The degree of humidity in the air is of considerable importance, and here again a wide range is possible, from 40 to about 80 per cent relative humidity being tolerated but 55 to 65 per cent giving good results.

*Continuous Breeding*—The life cycle from egg to death of the female is about forty-five to fifty days. Under favorable conditions in the laboratory, it is possible to have continuous breeding throughout the year. To keep the production of maggots unbroken it is best to reserve some of the eggs laid early in the life of the female and allow them to develop into another generation when it is necessary to replace the old brood. Under the treatment recommended here, there will probably be no trouble in producing succeeding generations in winter. Inbreeding is apparently not detrimental to egg production, but it may be advisable to mix with a strain from another laboratory every few months.

#### GENERAL METHODS OF REARING

*The Rearing Cabinet*—For the successful production of maggots and flies throughout the year an incubator or rearing cabinet is practically essential. Such a cabinet can be made at comparatively low cost. It may be constructed of wood, with two glass doors in front opening outward and glass in each end. A shelf allows two layers of cages to be used. The inside dimensions of the cabinet will depend upon the number and size of the cages.

The heat required for the cabinet may be supplied by electric-light bulbs or coils of resistance wire, placed at one end of a compartment below the cabinet. The heaters are connected to a heat regulator in the cabinet. Regulators of various kinds are listed in catalogs of scientific apparatus.

At the other end of the lower compartment a humidity chamber is constructed. There are several devices available for this purpose. Satisfactory results can be obtained with a shallow, broad water pan designed to allow lengths of absorbent cloth to hang down into the water. An electric fan placed between the heaters and the water pan draws air through an opening in one end of the compartment over the heaters and blows the warmed air across the moist surface of the cloths. The air then goes upward through openings in the floor of the cabinet, and after circulating leaves the cabinet through small openings in the sides near the top. The degree of humidity can be varied with the extent of moist cloth and with the temperature of the water in the pan.

Disagreeable odors should not occur in the production of sterile maggots for use in wounds, but in rearing maggots through to the pupal stage considerable unpleasant odor will develop. It is best, therefore, to use a separate cabinet for this purpose and to draw off the odors through an exhaust fan at the top which is attached to an outside vent.

*The Fly Cage*—Several types and sizes of cages have been used for the adults. The small, cylindrical, cloth-covered cage described by Simmons<sup>17</sup> has been found most satisfactory here. It consists of a circular base and top of wood, the top having a large circular opening and braced beneath to prevent cracking. The sides are metal rods threaded at top and bottom and held in place with two nuts at each end. The dimensions of the cage are 14 inches high and 10 inches in diameter. A cloth sack of mercerized lawn is made to be pulled over the cage and fastened at top and bottom with a cord in a slot cut around the edges. The sack is made with a short sleeve which provides access to the cage. The top is covered with bronze wire gauze. The cloth cover becomes soiled in a few days and should be replaced, and the cage also requires cleaning occasionally. Flies can be easily transferred to a clean cage by inverting the clean cage over the soiled one and removing the intervening gauze. Transference is hastened by darkening the lower cage.

*The Maggot-Rearing Container*—Larvae are reared best in glass containers, and various shapes and sizes have been used. In rearing through to the adult stage excellent results have been obtained here with 600 c c beakers covered with two layers of cheesecloth. In the production of sterile larvae for use in wounds a special type of container is recommended and is described later. Larvae to be reared to the fly stage require a suitable place to pupate. A satisfactory method is to use a pan with about three quarters inch of sterilized sand in the bottom. When the maggots become nearly full grown, the container, with top removed, is placed in the middle of the pan. The maggots will leave the container and go into the sand to pupate.

*Feeding Technique*—Flies. The food dishes, previously described, are placed in the cage through the sleeve. When honey-yeast is used, the food-pad tends to dry out and should either be replaced daily or well moistened with water on the second and third days and replaced on the fourth day. The meat also dries and should be replaced daily.

*Maggots*. For stock rearing, sufficient meat is placed in the beaker to last the larval period. Little further attention is required until just before wandering

commences, when the beaker should be placed in the pan. Food for sterile maggots is described later.

*Removal and Care of Eggs*—The eggs are deposited upon the meat, usually on the lower surface, in the fly cages. Meat having egg clusters should be removed in the Petri dishes from the cages at the end of each day and the dishes covered to prevent drying out. They should then be placed in the refrigerator to prevent hatching during the night. No meat should be left in the cages overnight because of the short hatching period of the eggs. In the morning most of the eggs are removed with a scalpel from the meat, and transferred to water for the sterilizing process. The meat with the remainder of the eggs may be placed in larva beakers to develop into stock flies.

*Procedure in Establishing Colonies*—1 The necessary incubators should be constructed and thoroughly tested.

2 Fly cages and other equipment should be ready for use.

3 About 100 to 150 pupae should be placed in each cage and put in the incubator. Upon request, the Bureau of Entomology in Washington will supply a sufficient quantity of pupae of the species *Lucilia sericata* to start two or three colonies.

4 The food for adults should be placed in each cage as soon as emergence occurs and the meat should be added two or three days later. The flies will begin to lay eggs in five to ten days.

5 To build up sufficient colonies for the required egg production, a large number of maggots should be allowed to develop into flies before an attempt is made to use eggs for sterile maggots.

#### THE PRODUCTION OF STERILE MAGGOTS

Maggots for use in wounds are reared under sterile conditions. On account of the possibility of serious infection from this source, it is strongly advised that the strictest bacteriologic technic be employed in this work. Sterilization is done in the egg stage, and the maggots are kept from contamination by the use of sterilized containers and food. Aseptic technic must be practiced throughout the entire process.

The eggs, which are usually laid in clumps, must be separated before sterilization is attempted. Baer<sup>3</sup> suggests vigorous stirring in water, Murdoch and Smart<sup>14</sup> use Dakin's solution, and Child and Roberts<sup>4</sup> use sodium hypochlorite solution.

Various methods and formulae have been recommended for sterilizing the eggs.<sup>1, 11, 21</sup> White<sup>21</sup> has obtained favorable results with a solution consisting of mercuric chloride 0.25 gm, sodium chloride, 6.5 gm, hydrochloric acid, 1.25 cc, ethyl alcohol, 250 cc, distilled water, 750 cc. The eggs are immersed for fifteen minutes. The formulae used by Murdoch and Smart and also by Baer are somewhat similar to that of White. Child and Roberts have used a three minute immersion in a "solution containing 4 per cent formaldehyde." White has also used formaldehyde and states that a 5 per cent solution (of formalin) gives good results.

After sterilization the eggs must be thoroughly washed. A method suggested

by both Baer and White has been found convenient. It consists in transferring the eggs to cheesecloth in a Gooch crucible supported by a wide-mouthed bottle, and washing several times with sterile water.

The next step is to remove the eggs to the larval food vial. This can be quickly done by placing the cheesecloth containing the eggs directly in the vial.

Convenient food containers can be made from shell vials about 80 mm high and 30 mm wide or wide-mouthed bottles of similar size. The food may consist of pieces of liver in nutrient agar and should be sterilized in an autoclave. Food to the depth of 10 mm is sufficient to carry the larvae over to the stage at which they are to be used in the wound. After the eggs hatch the gauze is removed from the vial.

On the second day after hatching, each lot of maggots is tested for sterility, with respect to both aerobes and anaerobes. Cultures are obtained from the partially liquefied food upon which the maggots are feeding. The maggots are then placed in the refrigerator to prevent development while the cultures are being incubated. Any vial of maggots which later shows contamination is discarded. At the completion of the sterility tests the maggots are still quite small, from 5 to 8 mm in length. The small size is best suited for use in the wound, as the maggots feed for only a few days at the most.

#### ENTOMOLOGIC ASPECTS OF THE MAGGOT TREATMENT

*Implantation of Maggots*—Sterile maggots are placed in the wound two to five days after the operation to remove devitalized bone has been performed. Baer,<sup>3</sup> Myers and Czaja,<sup>1\*</sup> Child and Roberts,<sup>4</sup> and Hewitt<sup>10</sup> describe the surgical procedure in cases for maggot treatment, and there is an abundant literature dealing with routine procedure in the operative treatment of osteomyelitis. Several methods have been used in transferring the maggots from the container to the wound. A simple method developed by White is to pour saline into the larval food vial, strain the maggots with gauze in the same way as was done with the sterilized eggs, and then transfer the larvae from the gauze directly to the wound. Aseptic technique must, of course, be used. Maggots received in vials free from food and ready for use may be removed conveniently with a cotton-tipped wood applicator.

The number of maggots used will depend upon the size of the wound, but will probably be from 200 to 600. Overcrowding should be carefully avoided. The maggots grow rapidly after implantation, and if too many are used they will shortly crowd each other and force some to leave the wound and crawl over the skin and get in the bed. It is best to use only as many as can be accommodated in the wound when they are full grown.

*The Use of Cages Over the Wound*—To confine the maggots within the wound, cages of several kinds have been used. Hewitt, however, has obtained satisfactory results with no cage at all in certain cases. When first placed in the wound, some of the maggots will tend to wander. Hewitt found that if the wound is fanned briskly the maggots will be cooled by evaporation and go into the wound for warmth. They may also be pushed back with an applicator. The patient himself can do this. Any persistent wanderers may be removed. Feed-



ing will soon begin and the maggots will arrange themselves side by side with heads downward

*No Danger of Parasitism*—The maggots become full grown in from four to five days after implantation. They then cease feeding and are of no further use in the wound. In nature the maggots leave their feeding place and migrate until they reach a suitably dry and dark place for pupation. Therefore, with the species *Lucilia sericata* recommended and supplied by the Bureau of Entomology, there is no danger of parasitism within the patient or of injury to the tissues through neglect to remove the maggots. However, as pointed out in a circular issued by the Bureau of Entomology,<sup>10</sup> there is grave danger in the use of the screw worm (*Cochliomyia macellaria* Fab.) and that species must be carefully avoided. Before the end of the feeding period the maggots should be removed, otherwise they will leave the wound when feeding is finished and cause considerable annoyance to the patient.

The attitude of patients to the use of maggots is usually one of tolerance and cooperation. During the early stages of treatment, the maggots sometimes cause sharp, intermittent pain within the wound. Also an abundant drainage from the wound occasionally irritates the skin, and a brief rise in temperature may occur. These and other medical aspects of the treatment are discussed in the articles already referred to.

*Number of Implantations*—After each removal of the full grown larvae from the wound, the patient may be given a day to rest. After several implantations, the irritating discharge usually changes to a copious serous discharge which does not inflame the surrounding skin. The number of implantations will vary with the individual cases, but are usually continued for six to eight weeks.

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## EMBEDDING CURETTINGS FOR FROZEN SECTION\*

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THE demand for a rapid diagnosis on surgical specimens has made the frozen section method decidedly popular with surgeons. Curettings from the uterus are frequently so small that it is difficult for the pathologist to make a frozen section. It is essential, however, to make a diagnosis as soon as possible. The usual methods of embedding in paraffin or celloidin require too much time and in most cases cannot be used.

The method which I have used for a number of years and which has proved satisfactory in my hands is as follows:

The fragments of tissue are spread out in a shallow dish (stender), covered with 10 per cent melted gelatin and allowed to harden. (This may be hastened by placing in the refrigerator.) As soon as the gelatin is hard the dish is placed in a jar containing a few drops of 40 per cent formaldehyde where it is allowed to remain about twelve hours for fixation of the tissue. It is then ready to be frozen and cut. The cut sections are placed in cold distilled water, floated on a slide, which has been smeared with egg-albumen, the excess water is drained off and the section covered with a cigarette paper and blotted. By carefully removing the cigarette paper the section may be run through any staining method desired.

The advantage of this method is that the smallest fragments may be examined easily and quickly.

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## ROUTINE BACTERIOLOGIC DIAGNOSIS OF DIPHTHERIA SWABS BY MEANS OF CLAUBERG'S BLOOD TELLURITE MEDIUM\*

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SINCE the introduction of Loeffler's serum<sup>1</sup> as a medium for the bacteriologic diagnosis of diphtheria, there have been many attempts to perfect a differential medium which might be worthy of substitution. The fact that salts of tellurium may exert a differential antiseptic action on various organisms has been known for over twenty years. Potassium tellurite and tellurates have been used especially in connection with various differential media for the bacteriologic diagnosis of diphtheria since it has been found that *C. diphtheriae*, and the diphtheroids in general, resist given concentrations of tellurites and tellurates more than the staphylococcus and colon typhoid group. The composition of the many tellurite media proposed for differential purposes has involved essentially only a difference in the enriching factors, the amount of tellurium salt being approximately the same in each case.

Among the earliest media proposed, which contained for their enriching factors serum and peptone water, were those of Conrad and Troch,<sup>2</sup> and Smith.<sup>3</sup> The former consisted of 0.02 per cent calcium tellurate with calcium bimolate in a medium very similar to that of Loeffler. The medium proposed by Smith differed from this in that approximately 0.01 per cent potassium tellurite was added to the horse serum peptone water agar. The main disadvantage to both these media lay in the fact that the growth of streptococci was entirely unrestricted. Pergola<sup>4</sup> devised a liquid transparent medium also using serum but with the addition of egg yolks for further nutritional purposes. An improvement over plain serum for enrichment was proposed by Douglas.<sup>5</sup> His tellurite medium contained sterile trypticized serum which was considered an improvement over plain serum in that it was found that complete or partial neutralization of antitryptic power increased the value of serum for cultural purposes. This was further improved upon by Allison and Ayling<sup>6</sup> who added 0.05 per cent copper sulphate to the usual tellurite agar medium. By this means staphylococci, streptococci, pneumococci, *N. catarrhalis*, and *B. proteus* were ruled out and the differential characteristics of *C. diphtheriae*, *C. hoftmanni*, and *C. velosis* were more accentuated. The copper sulphate apparently had no effect on the virulence of the diphtheria organisms. Finally whole blood was successfully substituted for serum in the usual tellurite media. In 1929 Clauberg offered his cooked blood aseptic fluid tellurite medium and in 1931<sup>7</sup> modified it to a simple medium containing equal volumes of blood-glycerine, tellurite, agar and liked sheep's blood. Anderson, Happold, McLeod and Thomson<sup>8</sup> also proposed a chocolate tellurite medium containing sterile meat infusion which had never been heated over 75° C. Differentiation by this medium was considered more successful than by Loeffler's serum slants. Using a blood cystine enriching

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base for tellurite agar Pesch and Kramer<sup>10</sup> reported excellent differential results after the addition of copper sulphate

However, of all the tellurite media proposed, that of Claueberg<sup>7</sup> has been most favorably commented upon Among the many who have reported success with it have been Manzini,<sup>11</sup> Tempe,<sup>12</sup> Seistrop,<sup>13</sup> and Tallo<sup>14</sup> Up to the present time results of actual use of Claueberg's modified medium<sup>8</sup> have not been reported in detail Mention of its existence and purported success was made in the report of Anderson and his colleagues but details of its actual adoption are still lacking

The simplicity of the medium and the success reported by Claueberg prompted the following test of its practicability for the routine testing of diphtheria swabs as compared to the usual staining of growth from Loeffler's serum slants

#### THE MEDIUM

The medium was made precisely according to the directions given by Claueberg in his report of 1931<sup>8</sup> Care was taken to use fresh ingredients only The formula was as follows

##### A Blood mixture

- 1 One part sterile blood (fresh sheep or beef)
- 2 Two parts sterile distilled water

##### B Blood glycerine agar

- 1 Two parts sterile sheep blood
- 2 One part sterile glycerine
- 3 After six to eight weeks or more, mix aseptically the glycerine blood in 5 per cent concentration with 3 per cent nutrient agar P<sub>H</sub> 7.5 at a temperature between 45° and 50° C

Add equal parts of mixture A and freshly made still fluid mixture B, plus four parts in a hundred of 1 per cent stock potassium tellurite Pour the plates not over 5 mm thick Make fresh tellurite solution for each batch of medium Eimer and Amend, C P potassium tellurite was found to dissolve readily upon warming the solution and further steps were never found necessary for its preparation It was found advisable to prepare enough plates for not more than one week's supply, the stock being kept in the refrigerator until used

The actual expense involved in making each type of medium could not be calculated for it would depend to a great extent upon the facilities of the laboratory However, the cost of the basic ingredients\* might be considered standard everywhere Both the beef serum obtained from the slaughter house and the sterile sheep blood could always be obtained without cost

The comparative expense connected with the production of Loeffler's and Claueberg's mediums was found to be practically the same, the only difference being in the amount of labor and in the number of plates and slants which could be produced from an equal volume of each type of medium One hundred cubic centimeter volumes produced 50 to 100 Loeffler slants and 12 to 24 Claueberg plates depending upon the size of the test tubes and Petri dishes used

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\*Potassium phosphate (Dibasic) C P Baker's Analyzed  
 Potassium tellurite Eimer and Amend  
 Glycerine purified (U S P X)  
 NaCl, C P Baker's Analyzed  
 Dextrose, Merck  
 Peptone (Bacto) Difco  
 Beef extract, Liebig's

Substitution of sterile rabbit blood was found to be unsatisfactory since the *C diphtheriae* grew with noticeably less luxuriance than when sheep blood was used

## METHOD OF PROCEDURE

Through the kindness of Dr W H Kellogg, Director of the State Bacteriological Laboratory of California, fresh swabs were available for the routine tests. Each swab was first smeared directly on Loeffler's serum slants then streaked on a Clauberg plate. All plates were examined after twenty-two to twenty-six hours' incubation at 37° C. The diagnosis of the plates was made solely by eye from the macroscopic appearance of the colonies in strong daylight. These results were compared with those obtained by staining a smear of the swab planted simultaneously on a Loeffler slant. In cases where the results differed or were doubtful the virulence of the strain was determined by the method proposed by Force and Beattie<sup>15</sup> for the routine testing of field cultures from suspected diphtheria cases. Also the fermentation reactions of the significant carbohydrates, i e., glucose, lactose, sucrose, dextrin, galactose, maltose, and mannite, were tested in Hiss serum water. From each culture diagnosed as positive on the Clauberg plates the pure strain was obtained and checked both for typical morphology by staining with acetic toluidin blue and by the usual fermentation reactions.

It was found that examination of the plates later than twenty-four to twenty-six hours was unreliable for after that time *C hoffmanni* and a certain strain of *staphylococcus* would develop. These colonies appeared enough like those of *C diphtheriae* to cause confusion in their identity. The size of the typical *C diphtheriae* colony was found to vary in a marked degree, ranging on an average from  $\frac{3}{4}$  mm to 1 mm and sometimes more in diameter. In practically all cases the *C diphtheriae* was found to develop as a round smooth shiny and raised colony. By transmitted light the center of the young colony appeared as dark greyish-black extending over approximately one-third the whole area and becoming lighter towards the periphery. After twenty-four hours practically the entire colony became black. The diphtheroids, when they did appear, could, after some experience, be detected by the fact that the dark centers spread over a smaller area, were

TABLE I  
SUMMARY OF RESULTS

	509 SWABS TESTED							
	ORIGINAL DIAGNOSIS				FINAL DIAGNOSIS AFTER FURTHER TESTS FOR IDENTIFICATION			
	LOEFFLER SLANTS		CLAUBERG PLATES		LOEFFLER SLANTS		CLAUBERG PLATES	
	NO	PER CENT	NO	PER CENT	NO	PER CENT	NO	PER CENT
Positive	39	11.59	70	13.75	35	10.80	72	14.14
Questionable	2	0.39	8	1.57	0	0	0	0
Negative	448	88.41	431	85.68	454	89.20	437	85.86

blackest and the periphery did not have the characteristic whitish tinge of true diphtheria colonies. Some diphtheroid strains produced no dark centers at all. The streptococci, while developing clear cut dark centers, were not confusing because they were always flatter and more translucent than the C diphtheria colonies and usually developed later. As noted by Claueberg and others there was one strain of staphylococcus which was not inhibited in its growth. However, it was found that this strain could be detected, after careful scrutiny, since the dark center extended further and the periphery was more transparent than that in the strains of C diphtheriae colonies which grew typically large. In practically all the plates diagnosed as positive C diphtheriae there were found to be no contaminating organisms. In over half the remaining plates in which C diphtheriae was reported as negative no growth of any kind appeared.

## RESULTS

The diagnosis made by culturing 509 swabs simultaneously on Loeffler slants and on Claueberg plates yielded the following comparative results, shown in Table I.

Examination of the tabulated summary in Table I reveals that prior to any confirmatory tests 11.59 per cent of all the cases tested were positive by Loeffler's

TABLE II  
ANALYSIS OF FINAL RESULTS

	ACTUAL NUMBER	PER CENT	
		BASED ON TOTAL NO OF CASES TESTED = 509	BASED ON TOTAL NO OF POSITIVE CASES FOUND = 67
Total number of swabs diagnosed as positive (Not including questionable cases)	67	13.16	100.0
Total number of swabs diagnosed as positive by both methods	53	10.41	79.10
Total number of swabs diagnosed as positive by Claueberg plates but not on Loeffler slants	13	2.55	19.40
Total number of swabs diagnosed as positive by Loeffler slants but not on Claueberg plates	1	0.19	1.49
Total number of swabs diagnosed as questionable positives on Claueberg plates only	8	1.57	11.94
Of which proved actually positive	4	0.78	5.97
Total number of swabs diagnosed as questionable positives on Loeffler slants only	2	0.30	1.22
Of which proved actually positive	1	0.18	1.49
Total number of proved errors by Loeffler's slants Five cases diagnosed negative but proved positive Nine cases diagnosed positive but proved negative	14	2.74	20.88
Total number of errors by Claueberg's method Three cases diagnosed positive but proved negative One case diagnosed negative but proved positive	4	0.78	5.97

slants and 13.75 per cent by Clauberg's plates. However, owing to questionable positive cases by both methods and to contradictory diagnoses in certain cases further tests for virulence and carbohydrate reactions were made upon the cultures in question. The correction of the mistakes and the confirmation of the questionable cases changed the final numbers of *C. diphtheriae* cases detected by Clauberg's and Loeffler's methods to 10.80 per cent and 14.14 per cent of all cases tested respectively.

Inspection of results shows that 67 swabs or 13.16 per cent of all the cases tested by one method or the other proved to be *C. diphtheriae*. However, only 53 of these positives were found to be so by both methods. Thirteen of the remaining 14 swabs were detected by examination on Clauberg's medium and were completely overlooked on Loeffler's slants. Only one strain of true *C. diphtheriae* which was reported on Loeffler's medium was not recognized on a Clauberg plate.

#### DISCUSSION

As may be seen from Table II the errors made from the macroscopic findings on Clauberg's medium amounted to 5.97 per cent of all the positives recognized by both methods or 0.78 per cent of all the swabs tested. These incorrect diagnoses consisted of (1) Three swab cultures which were mistaken for *C. diphtheriae* but which proved to be negative by further tests, and (2) one swab culture considered negative but which was found to be positive later.

These four cultures all proved to be avirulent and by further confirmatory tests yielded the following results: (1) A streptococcus erroneously diagnosed as positive *C. diphtheriae* on a Clauberg plate; (2) A bipolar beaded rod with carbohydrate fermentations not typical of *C. diphtheriae* and diagnosed as positive *C. diphtheriae* on a Clauberg plate; (3) A solid rod with carbohydrate fermentations not typical of *C. diphtheriae* and diagnosed as positive *C. diphtheriae* on a Clauberg plate; (4) A bipolar rod with carbohydrate fermentation reactions typical of *C. diphtheriae* and with atypical growth on a Clauberg plate.

In each case the incorrect macroscopic diagnosis from Clauberg's medium was considered to be due to inexperience in recognizing *C. diphtheriae* on the plates before twenty-four hours' incubation had been completed.

On Loeffler slants the errors in diagnosis amounted to 20.88 per cent of all the positive forms found or 2.74 per cent of all the cases tested. These errors consisted of 14 cases, 5 of which were diagnosed as negative. On Clauberg's medium the growth was typical of *C. diphtheriae* and not only gave the correct carbohydrate fermentation reactions but were all virulent as well. The remaining 9 cases were diagnosed as positive *C. diphtheriae* from growth on Loeffler's slants but when cultured on Clauberg's medium yielded colonies giving the following results: (1) A solid rod which did not give the carbohydrate fermentation reactions typical for *C. diphtheriae*, growth atypical on Clauberg plate and found to be avirulent; (2) A solid rod with the fermentation reactions not typical of *C. diphtheriae*, atypical growth on a Clauberg plate and found to be avirulent; (3) A solid rod with carbohydrate fermentation reactions not typical for *C. diphtheriae*, atypical colony on a Clauberg plate and found to be avirulent; (4) A bipolar rod, giving no carbohydrate fermentation reactions typical of *C. diphtheriae*, atypical colony on a Clauberg plate and avirulent; (5) (6) Two cases in which but scant growth could be obtained after forty-eight hours and which proved to be streptococci only; (7) (8)

(9) All failed to grow on Clauberg plates both cultured directly from the original swab and also from the original growth on Loeffler's slants

It is true that these 9 cases which gave negative results on Clauberg's medium are proof of error by the Loeffler method in a negative way only, for the original Loeffler swab was not tested for virulence. Experience has shown that true *C diphtheriae* will develop on Clauberg plates regardless of how heavily contaminated the material and for this reason it has been felt that these results should not be disregarded

The 8 swabs which resulted in questionable growth on Clauberg's medium were diagnosed as typically negative by the staining of growth on Loeffler's slants. Further examination of isolated colonies from the plates proved that 4 of these were virulent *C diphtheriae* strains yielding typical fermentation reactions on the carbohydrates. Each strain appeared to have typical bipolar rods after growth on Loeffler medium. The 4 remaining cultures all proved to be avirulent, comprising (1) a barred club form with atypical sugar reactions, (2) a strain resembling *C hofmanni* morphologically and by the sugar reactions, (3) a solid resembling atypical sugar reactions, (4) a strain which was beaded and bipolar and which gave the typical reactions of *C diphtheriae* on the carbohydrate tests.

On Loeffler's slants only two cultures were found to be questionable *C diphtheriae* and to require further tests to confirm the diagnosis. One of them was typically positive on Clauberg's medium and proved to be both virulent and to give the typical fermentation reaction of *C diphtheriae* on the carbohydrates. The other culture failed to grow on Clauberg's medium.

#### CONCLUSIONS

1 In spite of the fact that more questionable cases occurred on Clauberg's medium than on Loeffler's medium the total number of actual *C diphtheriae* detected solely by eye was significantly greater by the former method than by the latter. It may be concluded that substitution of the macroscopic method of colony identification for the usual microscopic examination of smears, saves time without impairing the accuracy of the diagnosis.

2 We consider that the success of macroscopic diagnosis by Clauberg plates for the routine diagnosis of diphtheria depends to a great extent upon one factor, namely, the experience of the worker. In our opinion the plates are useless to one who has never worked with them before. It is believed that sufficient experience may be gained after two or three weeks to enable one to diagnose typical plates with confidence and greater accuracy than by the usual stains of smears from Loeffler slants.

3 The only disadvantages connected with the mediums lie (1) in the difficulty of keeping a sufficient number of plates on hand, and (2) in the expense of keeping a supply of fresh sheep blood available. The latter would necessitate either the purchasing of fresh sheep blood at the time it was required or the maintenance of a sheep for bleeding purposes. The comparative cost of equal volumes of Clauberg's and Loeffler's mediums has been found to be approximately the same. It is suggested that small Petri dishes be used since large plates are unnecessarily wasteful of the medium unless two swabs may be streaked on one plate.

4 One of the chief advantages of the medium aside from the differential anti-septic action of the tellurite lies in the fact that isolation by streaking these plates



not only permits diagnosis by means of the usual staining procedure but also makes possible the study from pure cultures. The time involved is no longer than the usual routine procedure.

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## COMPARISON OF THE KOLMER MODIFICATION OF THE WASSERMANN AND THE KLINE YOUNG PRECIPITATION TESTS\*

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A COMPARISON of the Kolmer Wassermann, using two antigens, the Kolmer and the Acetone Insoluble, with the Kline Young Precipitation Test, on 3390 routine bloods gave the following results:

3041—complete agreements or approximately	89.4%
52—1 plus Kolmer and negative Kline or approximately	1.6%
96—negative Kolmer and 4 plus Kline or approximately	2.9%
141—negative Kolmer and 2 plus Kline or approximately	4.2%
23—2 plus Kolmer and negative Kline or approximately	0.8%
37—anticomplementary or approximately	1.1%
3390—Total number of cases	100.0%

\*From the Laboratories of the Mississippi State Hospital.  
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In the cases showing  $\pm$  plus Kolmer and negative Kline, using the Kolmer Modification of the Wassermann, the spinal fluids were as follows

17—positive or approximately	41.5%
24—negative or approximately	58.5%
11—No punctures	
52—Cases	

In those cases showing negative Kolmer and  $\pm$ -plus Kline, using the Kolmer Modification of the Wassermann, the spinal fluids were as follows

29—positive or approximately	36.3%
51—negative or approximately	63.7%
16—No punctures	
96—Cases	

#### CONCLUSIONS

1 The Kline reaction may appear to be slightly more sensitive in blood serology

2 In two small series in which there was a total disagreement between the Kolmer and the Kline Young reactions on the blood, there was approximately the same proportion of positive spinal fluids, using the Kolmer reaction as a basis

3 Both tests should be run as a routine procedure in the laboratory in order that no positive may escape

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#### A SIMPLE CONSTANT INJECTION APPARATUS\*

SAMUEL SOSKIN, M D , PH D , CHICAGO, ILL

WITH THE TECHNICAL ASSISTANCE OF HANS WILSON

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FOR some time we have been using a simple constant injection apparatus of our own design, activated by a windshield wiper of the pressure type. This apparatus is simple and economical in construction, accurate and positive in action, and has the further advantage that it automatically registers the amount of solution injected.

The apparatus consists of a  $\frac{3}{4}$  inch square bronze block,  $\frac{1}{4}$  inches in length, through the long axis of which a  $\frac{3}{8}$  inch hole has been bored. Through this hole a sliding-fit rod operates with a to and fro motion. The rod is fastened by a cross-member at each end to a guide bar, which slides through holes in the supporting posts of the square block. At each stroke of the windshield wiper a small crank attached to the windshield wiper shaft pushes a post on the guide bar. This causes the spring pawl at one end of the sliding rod to engage in the ratchet wheel. As the windshield wiper reverses its direction the sliding rod is returned to the resting position by a compression spring at its opposite end. An adjustable collar at the pawl end of the sliding rod determines its resting position and allows for the adjustment of the stroke. The ratchet wheel is supported by two pillar blocks and its center is finely threaded to receive a long threaded bar. A channel and pin prevent the threaded bar from rotating while the apparatus is in motion, so that the move-

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\*From the Metabolism Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.  
Received for publication April 1, 1932.

ment of the ratchet wheel causes the threaded bar to push the piston of the syringe. The pin may be released to allow the threaded bar to be quickly returned to its original position when the syringe is to be refilled. The syringe is firmly held in a divided wooden block held together by thumb screws. At each stroke of the windshield wiper a crank attached to its shaft operates a counting device.

Although this apparatus is particularly suitable for slow rates of injection, the injection speed can be almost infinitely varied by adjusting (a) the size of the syringe used, (b) the strength of the solution used, (c) the length of the stroke, (d) the speed of the windshield wiper (regulated by means of a needle valve). When great accuracy is required the syringe to be used may easily be calibrated by causing it to deliver into measuring flasks or weighing bottles. The amount of solu-

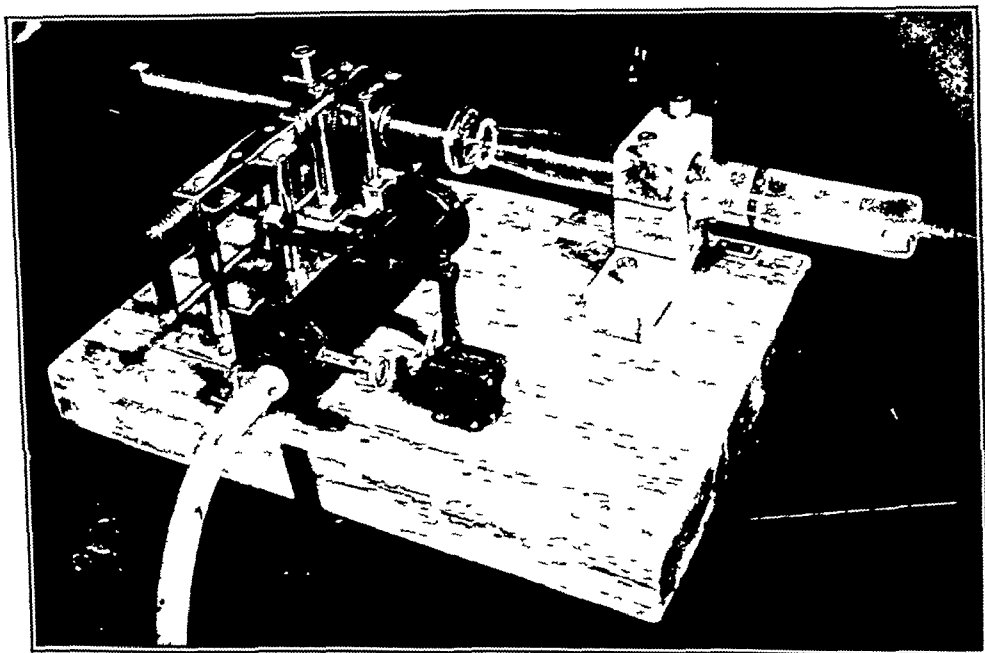


Fig 1

tion delivered at each stroke of the windshield wiper (recorded on the counting device) can then be calculated. We have used as our source of air pressure a compression pump and storage tank system, which delivers air at 30 to 35 pounds pressure depending upon the number of outlets simultaneously in use. With this source of pressure our injection apparatus has repeatedly run eight continuous hours without regulation, with a total error of about 2 per cent in the amount of solution injected in that period. By periodic timing and adjustment of the windshield wiper this error can be reduced to almost zero.

This apparatus has a distinct advantage for the injection of sterile solutions in that only the syringe and the tubing and needle attached to it need be sterilized. It can be made portable and hence used outside the laboratory by making use of tank of compressed gas to run the windshield wiper.

We wish to acknowledge our indebtedness to Fred Overthun for assisting in the construction of this apparatus.

## LABORATORY AIDS\*

RAYMOND H. GOODALE, M.D., WORCESTER, MASS.

EVERY laboratory develops certain conveniences which meet its particular needs. Many of these devices can be adopted by other laboratories with very little expense. The articles which are described here were developed in this laboratory as the needs arose. They have worked out very satisfactorily, and so I am passing them along to others.

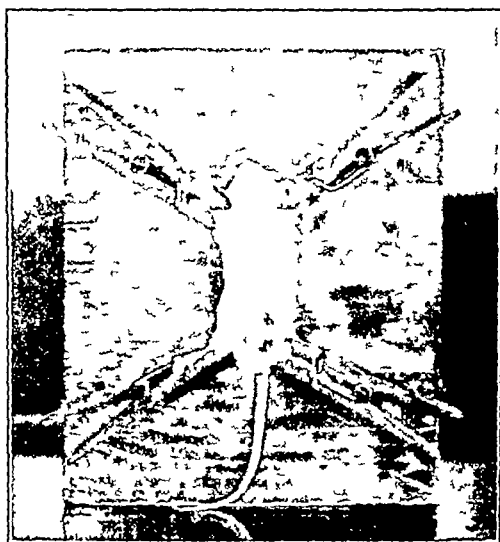


Fig 1—Mouse board

### 1 MOUSE BOARD

A simple and inexpensive mouse board was made by nailing four spring-type clothespins to a piece of board measuring 8 inches by 8 inches. The position of the legs of the average-sized mouse used is outlined on the board. The clothespins are then nailed so that the claws will be in the right position to seize the legs (Fig 1). The clothespins are nailed with a single nail through the spiral spring in the center. This allows the clothespins to be adjusted to the size of the animal. A larger type of this board can also be used for guinea pigs.

### 2 STAIN ILLUMINATOR

We have wasted considerable time in locating pieces of sectioned tissues in the dark stains such as hematoxylin and Geshichter's "metachrome 136". To avoid this loss of time we set the staining dish on the illuminating table seen in Fig 2.

\*From the Pathological Laboratory, City Hospital.  
Received for publication April 35 1932.

This is simply a square piece of window glass in a frame 6 inches by 6 inches and supported by four legs four inches high. The light is furnished by a 25 watt bulb fastened under the glass. This reduces the time consumed in staining and assures one of finding all of the pieces of tissue.

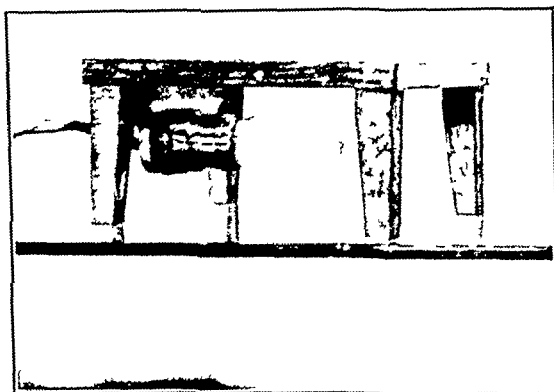


Fig 2—Stain illuminator

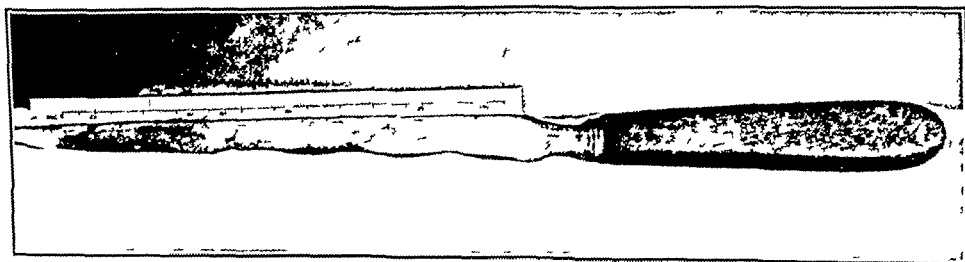


Fig 3—Measuring knife

### 3 MEASURING KNIFE

In describing surgical and autopsy tissues a ruler is necessary. In order to measure and cut tissues quickly the ruler and knife combination shown in Fig 3 was devised. It was made by cutting off the centimeter side of a 15 centimeter celluloid ruler and attaching it to the flat back of a knife with china cement.

## A DOG-MASK FOR RESPIRATION EXPERIMENTS\*

H F PIERCE, PH D , B ALTIMORE, MD

THE type of mask here described has been used by the author for various respiration experiments during the past ten years, and was designed to provide a simple means of making masks for all sizes of dogs, goats, and other animals having long snouts. The mask is made from a tin can, a short piece of one-half inch brass

Fig 1

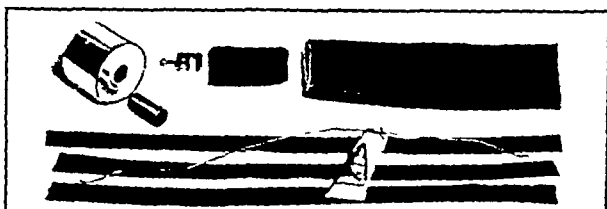


Fig 2

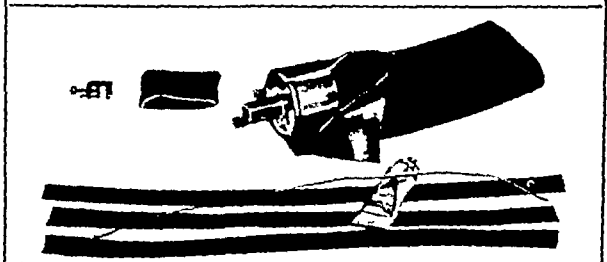


Fig 3

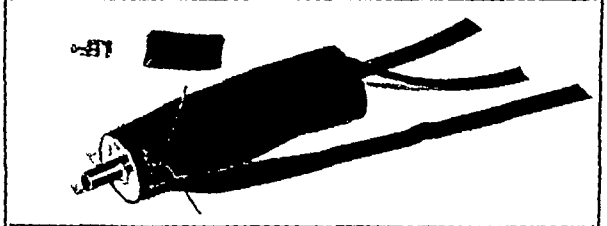
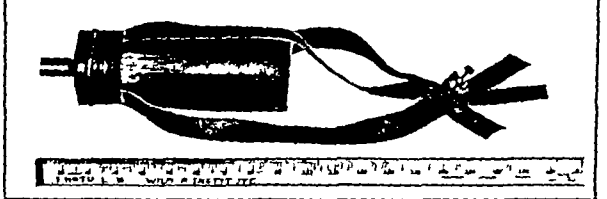


Fig 4



tubing, and a section of automobile tire inner tube (Fig 1). Food tins may be had in a great variety of sizes, and old inner tubes from 2 inches to 7 inches in diameter may be obtained at any tire repair shop. The inner tube should be chosen to fit the animal's nose tightly at a point just below the eyes and behind the corners of the mouth. The tin should be large enough not to press upon the sensitive parts of the animal's nose.

\*From the Wilmer Institute of the Johns Hopkins University and Hospital  
Received for publication May 4 1932

The tin is cut down to a length of 2 inches to 3 inches, a hole made in the end, and a short piece of brass tubing soldered into the hole, care being taken that no sharp projections remain inside the tin. If the inside of the inner tube is rough it should be turned inside out, so that the inner surface of the mask will be smooth. The inner tube is split lengthwise for a distance of 4 inches and the flaps sand-papered, treated with rubber cement, and wrapped tightly around the tin (Fig 2). A small opening between the tin and the rubber remains where the lap occurs, and should be filled with cement to make the joint air-tight.

Three strips 1 inch wide and 18 inches long are cut from an inner tube and cemented, at one end, to the mask, 120° apart (Fig 3). Two turns of No. 16 B and S gauge copper wire are wound tightly over these joints, and, last, a piece of inner tubing the size of the tin is slipped over the wire and cemented in place.

If the animal is not of the short-haired variety, it is advisable to clip the hair about the muzzle before applying the mask. The hair should then be rubbed full of heavy petrolatum and the mask slipped over the nose. The long elastic straps fix the mask in position. One passes between the animal's eyes and over the top of the head, and the other two along the angles of the jaw and under the ears to the back of the head, where the three are fastened together by means of a Hoffman clamp (Fig 4). After the mask is fitted and the straps drawn tight, any possible openings below the eyes or under the jaw should be filled with petrolatum. This has been found to maintain a tight joint up to a pressure of 1 inch of water.

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## A NEW METHOD OF MOUNTING EYES AND OTHER SMALL MUSEUM SPECIMENS\*

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ANDREW A. EGGSTON, M.D., NEW YORK, N. Y.

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THE following method for the mounting of museum specimen of eyes and small specimens have been used at the Manhattan Eye, Ear and Throat Hospital for a number of years and has proved of so much satisfaction as to justify its publication.

I. Fix the enucleated eye or specimen in Klotz solution after having made a small slit through the optic nerve in the case of an eye with a razor blade. Leave the specimen in this fixing solution for six to seven days.

II. Halve the eye with a razor blade starting from the slit originally made in the optic nerve, sectioning the eye downward by a short sawing motion, until the blade arrives at the level of the lens. Then seize the razor blade at each end firmly with thumb and index fingers of both hands and press down firmly until lens is severed and the globe is completely halved.

III. Place the half of eye to be mounted in 70 per cent alcohol for thirty minutes, 95 per cent for thirty minutes and absolute alcohol for a few minutes. Drain and fix on perforated celluloid plaque bearing name, number and diagnosis written with India ink. Fasten eye to plaque by means of a coat of celloidin around edge of hole. Let dry for several minutes.

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\*Received for publication May 10 1932

IV Immerse the celluloid sheet with the specimen attached in special jar filled with Kaiserling III and bend plaque so as to have upper edge of it press against neck of jar. Close with tight fitting cover and clamp. A metal clamp is attached to the jar which will support one slide of a mounted section of the eye, thus completing the record of the specimen.

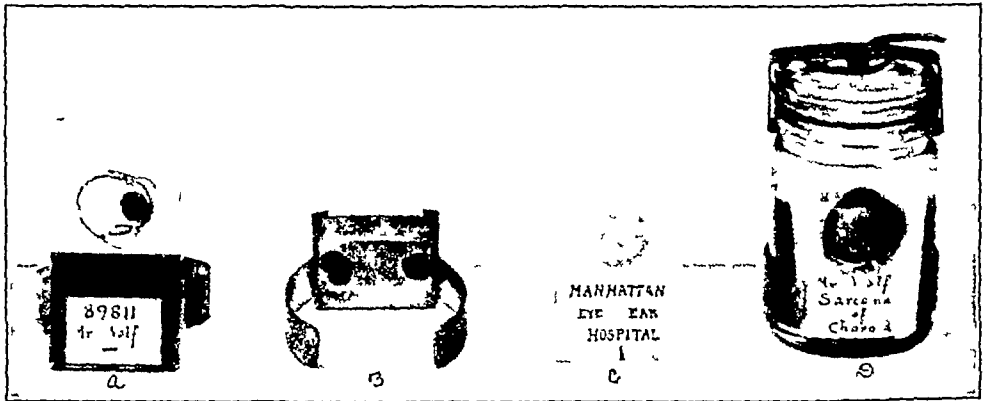


Fig 1—A Slide clip with slide in place and title B Slide clip C, Celluloid sheet with hole for specimens and title D, Eye mount complete

The advantages of this method consist of preservation of natural color. Immobile fixation of specimen to celluloid sheet in the jar so that the jar may be handled in a very rough manner even set upside down and the specimen remains undisturbed. No deterioration of medium and air bubble formation as occurs in the gelatin mount so long used in museum preparation of eyes.

The materials necessary for the mounting may be obtained from the Apex Scientific Equipment Co., 92 Monroe Street, New York City.



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**TISSUE** A Time Saving Procedure for Embedding in Paraffin, Baumgartner, W. J., and Welch, B. M. *Stain Techn* 7: 129, 1932

Short sections of rubber tubing are set on a glass slide, a piece of plain window glass an inch or more square, or the tube may be set into a watch crystal. The glass is rubbed with glycerin and the tubing cooled before using. The tubing is set upon the glass and warm paraffin run into the rubber container. The paraffin in contact with the glass and rubber cools slowly and hardens. Meanwhile, the specimen is placed and oriented in the center. Blowing a little air over the top will form a congealed film. The entire preparation is lowered carefully but quickly into cold water. As the paraffin cools it shrinks but the rubber tubing yields on all sides. Thus the shrinkage is not all from the top and so does not cause the "center dip" commonly resulting in solid containers. When fully hardened, the block is removed from the slide and the rubber separated from the round, well formed block of paraffin, which may then be labelled. The tubing and glass are again ready for use.

The size of the rubber tubing and the length of the sections to be used will vary with the size of the piece to be embedded. The thickness of the rubber may vary, the only essential being that the tube shall maintain its shape. The authors recommend tubing of 10 and 20 mm. in diameter, and sections cut 7 and 14 mm. in length. A good way to cut the rubber tubing so that the sections will have smooth, straight edges is to place it on a round rod of wood, which is then put into a lathe. The tubing is now cut readily into sections with a sharp knife while the wood is turning.

**BROMIDE IN BLOOD** Determination of, Finner, L. L. *Clifton Springs Med Bull* 18: 114, 1932

Whole blood, plasma, or serum may be used, and only 2 c.c. are required. The proteins are removed by the procedure of Fohn and Wu. The filtrate is treated with a knife point full of powdered animal charcoal, shaken for a minute, and refiltered. To 10 c.c. of filtrate is added 0.5 c.c. of 1 per cent gold chloride solution, and the color is compared after one minute and within 10 minutes with that obtained by treating 10 c.c. of bromide solution of appropriate strength with the same amount of gold chloride. Without the charcoal treatment, occasional filtrates develop a turbidity on adding the gold chloride. A stock standard solution containing 0.3 mg. bromine per 100 c.c. is convenient. From 1 c.c. to 10 c.c. of the stock solution are introduced into a series of test tubes, and the volume in each tube is made up to 10 c.c. Such a series covers blood bromine ranges from 30 mg. to 300 mg. per 100 c.c. The standard bromide solution should be titrated against silver nitrate.

At concentrations as high as 300 mg. bromine per 100 c.c. blood, the error does not exceed -10.0 per cent, and in the concentration usually encountered clinically, added bromide can be recovered quantitatively. When the concentration is less than 30 mg. bromine per 100 c.c. blood, the color becomes too faint for accurate comparison. Except for iodides, which also give a brown color with gold chloride, the reaction is apparently specific for bromides.

**B. TUBERCULOSIS** Tissue Substrate Microculture for Tubercle Bacilli, Corper, H. J. *J. A. M. A.* 99: 1315, 1932

The method consists of placing from 0.5 to 1 c.c. of well ground up or finely divided suspected specimen (sputum, tissue, etc.) in a sterile bacteriologic test tube, 6 by 3/8 inch, or 6 by 3/4 inch, stoppered with a cork after sterilization by dipping the end to be inserted into the tube into hot sterile paraffin. To the suspected specimen is added a few drops (0.5 c.c.) of sterile distilled (1/10 volume of sterile 3 per cent neutral trisodium citrate) human blood (other bloods

if available will also do) or 0.5 c.c. of fresh egg yolk. Blood appears more suited than egg yolk, primarily because of the more scattered skin colonies growing in the blood, although egg yolk has proved satisfactory as a nutrient in control tests. To the well mixed specimen and nutrient is added about from  $1\frac{1}{2}$  to 2 volumes of 6 per cent sulphuric acid which is intimately mixed by shaking, and the acid preparation is then placed in the incubator at  $37^{\circ}\text{C}$  for about forty five minutes (a range of from one-half to one hour is admissible), being vigorously shaken occasionally during incubation. After removal from the incubator, the acid is neutralized by the cautious addition of a volume of sterile 1.3 per cent pure sodium bicarbonate solution containing 3 per cent pure glycerin previously determined adequate to neutralize the amount of 6 per cent sulphuric acid used. Bromothymol blue, 0.04 per cent, is used as indicator and a deep green blue ( $\text{P}_{\text{H}}$  6.8) or light blue ( $\text{P}_{\text{H}}$  7.4) color is found to be satisfactory, following Clark's description and technique of making the tests. Neutralization can also be performed with sodium hydroxide, although the danger of passing the neutral zone is greater and it does not act as efficiently as sodium bicarbonate as a buffer during incubation. After neutralization, the tissue substrate is allowed to settle overnight in the refrigerator or the mixture can be centrifugated at low speed, after which the supernatant liquid is decanted, leaving a small portion behind (from 0.5 to 1 c.c. above the sediment) to serve in readily shaking and making smears when required, a 2 to 3 c.c. total volume of tissue substrate and liquid suffices when the original specimen including nutrient was from 1 to 1.5 c.c. in amount. After shaking to break up the sediment, the tube is carefully stoppered and is placed in a dark incubator at  $37^{\circ}\text{C}$ . After shaking the tubes well on removal from the incubator, smears are made at weekly or biweekly intervals on clean slides covered with a thin film of Meyer's albumin fixative, and after carefully fixing by heat, they are stained with steaming carbolfuchsin according to the Ziehl-Neelsen technique for acid fast bacilli, precautions being taken not to wash off the smear during staining and decolorizing.

**PLASMA-PROTEIN, Simple Test for Below The Edema-Producing Level, Page, I. H., and Van Slyke, D. D. J. A. M. A. 99: 1344, 1932.**

*Reagent*—Xylene, 1 volume, monochlorobenzene 2.06 volumes. One drop of a 3.36 per cent sodium chloride solution (Sp. grav. 1.0235) should neither rise nor fall but float in the mixture.

*The Test*—The freshly drawn blood is mixed with heparin, 1 mg. per cubic centimeter of blood, or with oxalate, not more than 2 or 3 mg. per centimeter, and is centrifugated. The clear plasma is dropped from a pipette held about 2 cm. above the level of the fluorobenzene or xylene-chlorobenzene mixture. If the plasma's specific gravity is below that of the fluid, the drop will rise rapidly to the surface, whereas if the drop has a higher specific gravity, it will fall rapidly to the bottom. Plasma just at the critical edema level will bob around if the tube is gently shaken, seemingly unable to decide whether to rise or fall. After a short time the drop flattens on the side or bottom of the tube and remains there as a record of its specific gravity.

**PNEUMONIA, A Study of Lobar Pneumonia in Massachusetts. Methods and Results of Pneumococcus Type Determination. 1931-1932, Heffron, R., and Varley, F. M. Am. J. Pub. Health 22: 1230, 1932.**

1. Pneumococcus typing is being requested by increasing numbers of physicians in Massachusetts.

2. During the past winter all 32 (Cooper) types of pneumococci excepting Types XXV and XXXII were found.

3. There was no especial geographical distribution evident of any of the types found.

4. Multiple cases of lobar pneumonia occurring in the same family at approximately the same time were very uncommon.

5. The Krumwiede, Sabin and tube agglutination methods of typing were and still are largely used and were found to be over 99 per cent accurate when definitely positive.

6. The Sabin method of typing has given a greater number of accurate positive typings in a shorter period of time than any other method studied, and is a method readily learned by technicians inexperienced in this work.

7. Of 789 specimens typed at the State Bacteriological Laboratory during the 11

months, September 1, 1931, to July 31, 1932, the type of pneumococcus found was checked in 94.6 per cent of the cases by a second method of typing or from a culture of the organism

8 The various sub types (Types IV to XXXII) were found more frequently during the 6 winter and spring months, December to May, than during the warmer months

9 In view of the results to date, and until conclusive proof to the contrary is obtained, it is felt that every type determination done by the Krumwiede, Sabin, or tube agglutination method should be checked by some other method of typing. Typing the organism obtained from cultures of the mouse heart's blood affords the most valuable means of checking

**ERYTHROCYTE VOLUME, Relation of Adrenal Glands to, Bon Haam, E., and Thatcher, H. S. Endocrinology 16 666, 1932**

From an experimental study the authors conclude that

1 The intravenous administration of adrenaline, asphyxia and bleeding produced a short lasting increase of the erythrocytes in the peripheral blood of 20 dogs. The autopsy revealed in all cases a marked contraction of the spleen during the time the hematocrit values in the peripheral blood were increased

2 Adrenalectomy prevented or hindered the reflex which leads to the splenic contraction and to the acute erythrocytosis in the peripheral blood after asphyxia and bleeding

3 It is concluded that the adrenal glands or their secretion, adrenaline, play an important part in the reflex mechanism which regulates the distribution of erythrocytes in the body

**SYPHILIS, Serology of From the Standpoint of the Public Health Laboratory, Kolmer, J. A. Am J Pub Health 22 1253, 1932**

The paper is summarized below

1 A choice of method or methods for the serum diagnosis of syphilis is a problem of special interest to public health laboratories

2 Of particular importance to all laboratories and especially those engaged in public health work without the advantage of skilful clinical checking of reactions is the question of the specificity of weakly and unexpectedly positive reactions

3 What is particularly required is the use of a test or tests possessing only the maximum of sensitiveness consistent with specificity in order to earn the confidence of the medical profession in the significance of weakly positive reactions

4 By employing a test or tests possessing the maximum of sensitiveness consistent with specificity, public health laboratories can do a great deal in minimizing the regrettable error of insufficient treatment of syphilis

5 Precipitation tests for syphilis are more economical than the Wassermann test since a hemolytic system is not required but they demand an equal degree of skill and are more subject to error and interpretation of weakly positive and doubtful reactions

6 Not infrequently the various precipitation tests for syphilis have been compared with complement fixation tests lacking in acceptable sensitiveness and specificity and consequently resulting in erroneous conclusions

7 Despite strenuous efforts none of the precipitation tests can be said to have displaced complement fixation tests possessing an acceptable degree of sensitiveness and specificity when judged impartially and scientifically with adequate clinical control. The use of two or more precipitation procedures has not proved as satisfactory as complement fixation and precipitation tests of acceptable sensitiveness and specificity for the routine testing of sera

8 It is impossible at present to make a choice of a single precipitation test from among the large number available

9 The new Marmice or "clarification" test has been found more sensitive than the Kahn test of equal specificity and easier to read and interpret the reaction

10 In a comparative study of the Kline microscopic and Kahn precipitation tests and the Kolmer modification of the Wassermann test, the reactions agreed in 86.7 per cent of over 1000 sera and disagreed in 1.3 per cent

11 In general terms the Khne microscope test was more sensitive than the Kahn and Kolmer tests but likewise gave a higher percentage of nonspecific or falsely positive reactions than the Kahn test while the Kolmer modification of the Wassermann test gave no falsely positive reactions

12 At the present time the serum diagnosis of syphilis is best served by conducting a carefully chosen complement fixation and precipitation test on each serum

**LIVER, Functional Capacity of Comparative Merits of the Five Most Popular Tests, Robertson, W E, Swalm, W A, and Konzelmann, F W J A M A 99 2071, 1932**

From a fairly extensive study the authors conclude that for routine procedure the icterus index is one of the most valuable tests for liver function The chief value of the van den Bergh lies in its selectivity for bilirubin, while the bromsulphalein test, especially when (in cases without jaundice) the 5 mg dose is used, most frequently agrees with the clinical picture

They were not impressed with either the galactose tolerance or urobilinogen tests

**BLOOD Study of "Iron Volume Index" and Its Significance in the Treatment of Anemia, Reich, C, and Tiedemann, V G Am J M Sc 184 637, 1932**

The following method is given for the determination of the new index proposed

Twenty cubic centimeters of blood are withdrawn from the arm vein and 10 c c are run into a 15 c c graduated centrifuge tube containing 2 c c of a 1.6 per cent sodium oxalate solution This is mixed by inverting, and centrifuged at 2500 rpm for one hour The total cell volume per cent is then read off and calculated for 100 c c

The other 10 c c are shaken up in a test tube previously prepared by evaporating 2 c c of 1.6 per cent iron free sodium oxalate solution to dryness The sodium oxalate used was obtained from the Bureau of Standards and shown by controls to contain no iron This blood is used for hemoglobin, red blood cell and iron determinations The hemoglobin was done by both the Sahli and Newcomer methods in each case, and 15.6 gm of hemoglobin were taken as 100 per cent Red blood cell counts were done with two standardized pipettes, a drop from each being counted for every determination, and the two averaged Results were not accepted if the error was more than 2 per cent

Iron is determined as follows

*Standard Iron Solution* *Standard A* Dissolve 0.702 gm ferrous ammonium sulphate in about 50 c c of water Add 20 c c of 10 per cent iron free sulphuric acid Warm and then add N/10 potassium permanganate until a faint pink color persists Dilute to 1 liter, 1 c c will contain 0.1 mg of iron *Standard B* Standard A diluted with an equal volume of water, 1 c c will contain 0.05 mg of iron

*Sulphocyanic Acid* One hundred grams of ammonium thiocyanate are dissolved in 100 c c of 65 per cent by weight sulphuric acid Measure volume, transfer to separatory funnel and shake out immediately with three fourths volume of amyl alcohol Discard the aqueous solution and again shake out twice with an equal volume of water Combine the water extracts, which together contain about 7 per cent of sulphocyanic acid Saturate with mercuric sulphocyanate so that some remains on the bottom Allow to stand over night in a dark bottle

*Persulphate Solution* A saturate solution of CP iron free sodium persulphate in distilled water

*Extraction Mixtures* (1) Five parts of amyl alcohol and 2 parts of ether, (2) equal parts of ethylene glycol monobutyl ether and ethyl ether

The ether does not have to be of special purity, as the presence of mercuric sulphocyanate prevents the oxidation of the sulphocyanic acid by the peroxides in the ether

*Iron Determinations* One cubic centimeter of blood is diluted with 4 c c of water and 1 c c of the mixture is digested according to the method of Wong After being allowed to cool it is diluted to approximately 10 c c and a drop of persulphate is added, followed by exactly 25 c c of the extraction medium and 5 c c of the sulphocyanic acid reagent The tube is then tightly fitted with a rubber stopper, the mixture is well shaken and after a few minutes' standing separates into two distinct layers If this separation does not take place

rapidly it can generally be accelerated by the addition of a few cubic centimeters of water. One cubic centimeter each of Standards A and B are then digested and treated in the same manner. The A Standard is suitable for figures within the normal range (35 to 50) and the B Standard is used where the iron values are lower than the normal. Since the A Standard contains 0.1 mg of iron and the B Standard 0.05 mg of iron, and 0.2 cc of blood are used, the formula for figuring the iron is as follows:

## FORMULA FOR FIGURING IRON

$$S \times \frac{R_s}{R_x} \times 500 = \text{mg of iron}$$

S = strength of standard

$R_s$  = reading of standard

$R_x$  = reading of unknown

Calculations

	Cv	Total cell volume, per cent	Normal Values
	46	Normal cell volume, per cent	46
Volume Index =	$\frac{RV}{RV}$	RBC in millions	46
	50	Normal RBC in millions	5
			5
	Hb%	100	
	100	100	
Saturation Index =	$\frac{Cv}{Cv}$	46	
	46	46	
	I	Iron in mg per 100 cc	45
	45	Average iron in mg per 100 cc	45
Corpuscular iron index =	$\frac{Cv}{Cv}$		46
	46		46

$$\text{Average volume of erythrocyte (Ev)} = \frac{Cv \times 10^{12}}{Rm \times 10^{14}} \text{ cubic micron} = \frac{46^{12}}{5^{14}} = 92$$

$$\text{Average Hb per erythrocyte (Hbe)*} = \text{Hb (gm)} \times \text{Ev} \times 10^{-14} = (15.6 \times 92) = 143$$

$$\text{Average iron per erythrocyte (Ie)*} = I \times \text{Ev} \times 10^{-14} = (45 \times 92) = 44$$

The normal figure for this index is between 0.9 and 1.0 and when this index is 1.0 the red cells are regarded as saturated with iron.

An iron volume index of 1.0 is obtained in most normal people and in cases of pernicious anemia, leucemia and in secondary anemias associated with carcinoma and acute and chronic infections with fever. Iron therapy seems to be of no value in these cases, due to the fact that the red cells are already saturated with iron.

An iron volume index of less than normal is most commonly found in cases of secondary anemia caused by malnutrition or loss of blood. Iron is of decided therapeutic value in the treatment of these patients.

## HEMOLYTIC SERA, Preparation of, Strafsseth, H. J. Science 76 444, 1932

The usual directions for the preparation of hemolytic and precipitating sera call for the use of washed red blood cells and serum respectively as antigens.

The fact is, however, that an immune serum prepared by using clear serum as antigen will function perfectly as a hemolytic serum and that a hemolytic serum prepared by using washed red blood cells as antigen will do very well as a precipitating serum.

\*In the table these determinations, namely average Hb and average iron are expressed as  $\times 10^{14}$  by pointing off three more places.

It is necessary to heat sheep serum for one half hour at 56° C and to remove fresh serum from the red blood cells by repeated washing in saline solution

Sterile serum or plasma can be kept for months or years, thus offering a saving in time and effort especially to those who may wish to work with hemolysins and precipitins without having ready access to sheep or goats

Giving rabbits 2, 3, 4 and 4 c c of sheep serum intravenously, allowing three to four day intervals between injections and eight to ten days between the last injection and the bleeding, has produced sera which when used as precipitins, showed titers of better than 1:2800. The same sera used as hemolysins dissolved 0.5 c c of a 2 per cent suspension of sheep erythrocytes in quantities of 0.01 c c of a 1 per cent dilution and of 0.05 c c of a 0.5 per cent dilution in the presence of 2 units of complement in thirty minutes at 37° C

Rather strong hemolytic and precipitating sera have also been obtained by using as antigen the clear saline solution in which blood cells had been washed, the solution from the fourth washing being about as effective antigenically as that from the first one

#### **SPIROCHETA PALLIDA** Staining of, Zingale, M. Policlinico Rome 39 1435, 1932

The following method is stated to distinguish spirochetes from pseudospirochetes

##### *Reagents —*

I Saturated alcoholic solution gentian violet	10 c c
Filtered amlin water	100 c c
II Neutral potassium carbonate	10 gm
Distilled water	200 c c

##### *Method —*

Place an arbitrary amount (10 to 12 c c) of Solution II in a watch glass and add for each centimeter used 1 drop of Solution I.

Prepare and fix a smear on a cover glass which is then inverted on the surface of the stain in the watch glass. Steam for 3 minutes and wash in running water.

The staining reaction is as follows:

Cellular detritus Bright violet on pearl gray background

Bacilli, cocci, and spirilla deep purple

Red blood cells bright lilac

Spirocheta pallida deep lilac like borders of erythrocytes

#### **SPINAL FLUID** Colloidal Benzoin Test, Evans, N., and Dodson, W. R. Am J Clin Path 2 463, 1932

From a study of 2000 reactions in 1800 cases the authors conclude that the evidence in the literature indicates that of the colloidal tests used in examination of the spinal fluid the benzoin test is the simplest to prepare and read, is as sensitive as and more informative than the gum mastic test, and is more sensitive than and as informative as the colloidal gold test.

A study of the data obtained from some 2000 colloidal benzoin tests on cerebrospinal fluids from 1800 patients signifies that (a) the benzoin test is not a specific test in the same sense as the Wassermann reaction, (b) it is of value in differentiating active general paresis from other forms of neurosyphilis, (c) it is probably of value in differentiating epidemic encephalitis from poliomyelitis and tuberculous meningitis, (d) a high percentage of positive readings is obtained in disease of the central nervous system, but a positive second zone reading is occasionally obtained in certain conditions not associated with organic disease of the central nervous system.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren I. Vaughan, Professional Building, Richmond, Va.

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### Recent Advances in Bacteriology<sup>1</sup>

THE recent advances series published by Blakiston constitutes a most laudable set of volumes. Covering, as it does the entire field of medicine and surgery, the volumes together constitute a very handy reference encyclopedia.

So much new has evolved within recent years in pure bacteriology and in clinical bacteriology that the man whose primary interest is not in this field finds it difficult to keep up with the advances or to find readily available within one volume in the literature a summary of the recent clinical advances that have been made. This volume fills that requirement.

Subjects discussed include such as the newer aspects of the streptococcus problem, bacterial variation, rough and smooth types, bacteriophage, Chalmers's BCG, ultramicroscopic viruses, the specific soluble substances of the pneumococci, haptens, antigen, Pasteur's local immunity, and the recent work on the anaerobes.

The volume should be of particular interest to clinicians who really want to keep abreast of what is happening in the field of bacteriology and epidemiology.

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### Recent Advances in Biochemistry<sup>2</sup>

THIS volume, another of the same series, is especially valuable since it does not start from scratch and saves all of its space for what are truly recent advances, thereby presupposing that the reader is well grounded in his fundamentals to start with. Subjects discussed include structure of proteins and proteolytic enzymes, amino acids, sulphur compounds and protein metabolism, the biochemistry of the fats, carbohydrates, vitamins, sterols, hemoglobin, and the chemical basis of specific immunological reactions. While the entire volume is given over actually to the discussion of the recent advances, the presentation is readily intelligible to one whose primary interest is not biochemistry.

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### Recent Advances in Medicine<sup>3</sup>

THIS volume, attempting as it does to cover an extremely wide field, necessarily does so with some sacrifice of detail. The subjects are all well discussed, but with many of them one cannot but regret that the author did not have more space to give them. It serves very well however as a general reference volume for the internist. In specific specialties other volumes in the series may be referred to.

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<sup>1</sup>Recent Advances in Bacteriology and the Study of the Infections. By J. Henry Dible, M.B. (Glas.) F.R.C.P., Professor of Pathology in the University of Liverpool, Late Professor of Pathology in the University of London, and Professor of Pathology and Bacteriology in the Welsh National School of Medicine. Second Edition. With 25 Illustrations. Pages 478. P. Blakiston's Son & Co. Inc. Philadelphia 1932.

<sup>2</sup>Recent Advances in Biochemistry. By John Fryde, B.Sc. (St. And.) M.Sc. (Wales), Lecturer in Physiological Chemistry, Welsh National School of Medicine, University of Wales. Formerly 1851 Exhibitioner in Chemistry. Third Edition. With 12 Illustrations. Cloth. Pages 331. P. Blakiston's Son & Co. Inc. Philadelphia 1931.

<sup>3</sup>Recent Advances in Medicine. Clinical Laboratory Therapeutic. By G. E. Beaumont, M.A., D.M. (Oxon.) F.R.C.P., D.P.H. (Lond.) Physician with charge of Out-patients, Middlesex Hospital, Physician to the Hospital for Consumption and Diseases of the Chest, Brompton, Medical Tutor, Middlesex Hospital Medical School. Sometime Padelisic Traveling Fellow, University of Oxford, and L. C. Dodds, M.V.O., M.D., Ph.D., B.Sc., Courtauld Professor of Biochemistry in the University of London, Director of Courtauld Institute of Biochemistry, Middlesex Hospital, Pathologist to the Royal National Orthopaedic Hospital. Sixth Edition. With 51 Illustrations. Cloth. Pages 412. P. Blakiston's Son & Co. Inc. Philadelphia 1931.

### Recent Advances in Allergy

**T**HIS is a case in point. While the volume on Recent Advances in Medicine devotes a few pages to Allergy, Bray's volume covers the clinical aspects of Allergy most comprehensively. It is written by a man who himself has made very laudable contributions to the field, particularly in his study of heredity and predisposition in Allergy, and the volume must be classed as paramount with the other recent works on Allergy by other authors.

### Recent Advances in Microscopy

**T**HIS volume is a series of monographs on the biological applications of microscopy as they are seen in medicine, the living eye, zoology, and botany. In the sections on medicine, botany and zoology, most of the discussion is given to the microscopy of the individual cell and this therefore provides a very good compendium of microscopic comparative anatomy. There is much on cellular reproduction. The section dealing with the eye is of particular clinical interest to ophthalmologists since it discusses the principles and the technique of the use of the recently developed slit lamp.

### Recent Advances in Pulmonary Tuberculosis†

**T**HIS is an especially adequate discussion in a relatively small volume. Subjects discussed include preventive vaccination, x-ray diagnosis (an abundance of good illustrations), sanatorium treatment, sanocrysin, pneumothorax, oleothorax, and the newer surgical methods of collapse.

### Recent Advances in Chemotherapy‡

**C**HEMOTHERAPY, conceived of by Paracelsus, is a science which has been developed by one man, Paul Ehrlich. Ehrlich's original conception based on his side chain theory was that certain chemicals might be hooked on to parasitic organisms thereby destroying their activity without being hooked upon the cells of the host. In the succeeding years this concept has had to be altered since it has been found that with the exception of emetin, certain anthelmintics and possibly Bayer 205, the action is not directly upon the parasite but is in direct through the reaction of the chemical with the body tissues.

The mechanism of parasitocidal activity appears to occur in either of four ways: (1) chemical interaction between the compound as administered or as transformed within the body, and some protoplasmic constituent of the parasite resulting in the death of the latter, (2) physicochemical interaction with the protoplasmic colloid of the parasites resulting in precipitation, coagulation or electrical changes sufficient to destroy or injure the parasite, (3) the production of new compounds within the tissues capable of chemical or physicochemical interaction with the protoplasmic colloid and (4) the production of antibodies within the body due to the release of antigenic substances from the parasites.

\*Recent Advances in Allergy (Asthma, Hay Fever, Eczema, Migraine, Etc.) by George W. Bray, M.B. Ch.B. (Sydney) Asthma Research Scholar, The Hospital for Sick Children, Great Ormond Street, London. With foreword by Arthur F. Hurst, M.A., M.D. (Oxon.), F.R.C.P. Senior Physician, Guy's Hospital, Chairman, Medical Advisory Committee, Asthma Research Council of Great Britain. 98 illustrations including 4 coloured plates. Cloth. Pages 432. P. Blakiston's Son & Co. Inc. Philadelphia, 1931.

\*\*Recent Advances in Microscopy, Biological Applications. Edited by A. Piney, M.D., M.R.C.P. Director of the Pathological Department, Cancer Hospital, London. Medicine by A. Piney, M.D., M.R.C.P. The Living Eye by Basil Graves, M.C., M.A., M.R.C.S. D.O.M.S. (Eng.) Zoology by D. W. McBride, L.D., D.Sc., F.R.S. and H. R. Hewer, A.R.C.S., D.I.C. M.Sc. Botany by E. C. Burton-Wright, M.Sc. With 83 illustrations. Cloth. Pages 260. P. Blakiston's Son & Co. Inc. Philadelphia, 1931.

†Recent Advances in Pulmonary Tuberculosis by L. S. T. Burrell, M.A., M.D. (Cantab.) F.R.C.P. (Lond.) Senior Physician to Royal Free Hospital, Physician to Brompton Hospital for Consumption and Diseases of the Chest, Consulting Physician to King Edward VII Sanatorium, Midhurst. Second Edition. With 32 plates and 17 text-figures. P. Blakiston's Son & Co. Inc. Philadelphia, 1931.

‡Recent Advances in Chemotherapy by G. V. Findlay, O.B.E., M.D., D.Sc., Wellcome Bureau of Scientific Research, London. With a foreword by C. M. Wenyon, C.M.G., C.B.E., M.B., B.Sc., F.R.S., Director-in-Chief of the Wellcome Bureau of Scientific Research, London. With 4 plates and 11 text-figures. Cloth. Pages 532. P. Blakiston's Son & Co. Inc., Philadelphia, 1930.



This volume reviews the development of our knowledge of specific chemotherapy and the changes in our interpretation of the process. It summarizes very concretely our knowledge of the action of the synthetic chemotherapeutic agents which are in use and many which are still in the experimental stage. It is surprising to observe how many of Ehrlich's original products which he more or less discarded are coming into favor usually under trade names. This is particularly true of the various amebicides and the hypnotics.

Particular attention is given to the chemotherapy of helminthic infection, protozoan infection and bacterial infection. There is also a chapter on virus diseases and one on cancer.

The value of the volume is not so much as a summary of recent work as it is an authoritative treatise on chemotherapy in general. There is only one other volume on chemotherapy available today, that by Kolmer which deals chiefly with syphilis. The two together make a very complete exposition of the subject.

### Injuries and Sport

FOR a thorough resumé of the injuries incident to sports, as well as the everyday routine, I do not believe a better work could be found.

The author has illustrated his examination, diagnosis, and treatment so efficiently with photographs and diagrams that the most difficult cases seem surprisingly simple. Although some of his methods are rather different from the generally accepted American ideas, as a whole the two are practically the same.

The most valuable material in the book is his common sense, practical use of splint and other forms of support.

Any physician or athletic director will do well to have this work in his collection.

### Statistical Methods for Research Workers

THAT this volume, No. V of a series of Biological Monographs and Manuals edited by F. A. E. Crew and D. Ward Cutler, has reached its fourth edition is ample indication of its usefulness to those to whom it is addressed.

The present edition is enlarged by the addition of several new sections and may be accepted as an authoritative presentation of the subject.

This book has been previously reviewed in this JOURNAL and it need only be said that the standard of previous editions is upheld by the present volume.

### Laboratory Manual of Physiological Chemistry†

AS implied by the title, this manual is not intended as a reference book, but is designed for the classroom.

It consists of an orderly presentation of well selected procedures designed to constitute a review of qualitative tests relating to physiological chemistry, and illustrative of the qualitative and quantitative procedures available for the analysis of biological material.

Of necessity the choice of methods has been to a definite extent influenced by their didactic value as well as the relative simplicity of the apparatus required.

The fact that the manual has passed into its second edition after a thorough practical trial in the teaching of students both at Johns Hopkins and the University of Pennsylvania is an indication of its usefulness.

For teaching purposes the volume may be recommended as a clear cut, concise, and eminently practical manual.

- M. I. \*Injuries and Sport. A General Guide for the Practitioner. By C. B. Heald. CBE. Oxford University Press. American Branch. New York. 1931.  
 †Statistical Methods for Research Workers. By P. A. Fisher. ScD. Chief Statistician. Potamsted Experimental Station. Ed. 4. cloth. 307 pages. Oliver & Boyd. Edinburgh.  
 †Laboratory Manual of Physiological Chemistry. By D. Wright Wilson. Benjamin Push. Professor of Physiological Chemistry. University of Pennsylvania. Ed. 2. cloth. 284 pages. Williams & Wilkins Co.

# The Journal of Laboratory and Clinical Medicine

VOL XVIII

ST LOUIS, MO, JANUARY, 1933

No 4

Editor WARREN T VAUGHAN, M D

Richmond, Va

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## EDITORIAL

Paul Gerhardt Woolley

ON THE title page of Volume I, number 1 of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE which appeared in October, 1915, we find the name of Paul G Woolley, editor for The Department of Physiological Pathology During the years of his active interest in medicine and medical research, Dr Woolley was one of THE JOURNAL'S most enthusiastic supporters and few numbers appeared without editorial contributions from his pen Following the war Dr Woolley found it necessary to take time off to combat an activated tuberculous infection to which he eventually succumbed at the Desert Sanatorium in Tucson on December 5, 1932

Paul Gerhardt Woolley was born in Paris, Illinois, April 7, 1875 He received his academic degree from The University of Chicago in 1896 and his M D from Johns Hopkins University Medical School in 1900 After serving as Resident House Officer at Johns Hopkins Hospital and Fellow in Pathology at McGill he became Bacteriologist, later Pathologist, and then Director of the Serum Laboratory of the Bureau of Science at Manila From the Philippines he went to Siam

where he became Director of the Siamese Government Serum Laboratory in 1906 and Chief Inspector of Health in Bangkok, in 1907

Returning to this country Dr Woolley accepted the chair of Pathologic Anatomy in The University of Nebraska but after a year he moved to Cincinnati where he became Professor of Pathology in The University of Cincinnati in 1909 For several years thereafter he was Dean of The Faculty of Medicine in The University of Cincinnati Medical School He was also Director of Laboratories and Pathologist to the Cincinnati General Hospital

While serving in Siam, Dr Woolley represented the Siamese Government at the International Congress of Tuberculosis in 1908 and at the International Congress on Hygiene and Demography in 1912 Dr Woolley lived for a time after the war in Detroit and in Los Angeles but found it necessary to give up his active work

During the war he held successive ranks from Captain through Lieutenant-Colonel in the Medical Corps and served as Instructor in Military Hygiene at Camp Greenleaf, Epidemiologist at Camps Greene and Devens, and Assistant Camp Surgeon and Sanitary Inspector at Camp Devens

Dr Woolley was the author of a volume, "The Clinical History in Outline" (1914), and of a number of papers dealing with subjects of pathology, bacteriology, tropical medicine, and animal parasitology He served as Associate Editor of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE from its founding to his death A host of friends join the editor and publishers in regretting the passing of a good fighter

—W T V

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## CORRESPONDENCE

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### To the Editor

In the article by Dr B S Levine in the October number, dealing with sensitizers other than cholesterol in Wassermann and flocculation antigens, he uses the term *sitosterol* to describe the sterol used in the flocculation technic recommended by me in a previous paper (JOURNAL OF LABORATORY AND CLINICAL MEDICINE, 17: 787, 1932). Strictly speaking, he is correct in that usage insofar as sitosterol is synonymous with phytosterol, the generic term covering all sterols of plant origin. However, some confusion may arise from the fact that the Difco Laboratories sell a specific sterol derived from wheat germ as sitosterol, while the corn germ sterol used in my flocculation antigen has entirely different sensitizing properties.

I originally recommended the wheat germ sterol (Bacto sitosterol) for use as an adjunct to cholesterol in a Wassermann antigen (*Journal Exper Med*, 53: 605, 1932). I have since found that the flocculation antigen, containing cholesterol and corn germ sterol, is equally serviceable as a Wassermann antigen, and have therefore abandoned the use of wheat germ sterol as an unnecessary complication. Contrary to the findings of Dr Levine, I find that maximal sensitization with sterols significantly increases the sensitivity of both tests, and in the case of the flocculation test adds greatly to the simplicity of the technic and clarity of the results.

(Signed) HARRY EAGLE

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### Erratum

In Dr Lepeschkin's article in the September, 1932, issue of the Journal, page 1251, under *distilled water*, in the second line of the tabular matter, 398 5 c c should read 308 5 c c

## ITEM

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The American Association for the Study of Goiter, for the fourth time, offers Three Hundred Dollars (\$300 00) as a first award, and two honorable mentions for the best three essays based upon original research work on any phase of goiter presented at their annual meeting in Memphis, Tenn, May 15, 16, and 17, 1933. It is hoped that this will stimulate valuable research work, especially in regard to the basic cause of goiter.

Competing manuscripts must be in English and submitted to the Corresponding Secretary, J R Yung, M D, 670 Cherry St, Terre Haute, Indiana, U S A, not later than April 1, 1933. Manuscripts arriving after this date will be held for the next year or returned at the author's request.

The First Award of the Hamilton, Ontario, Canada, 1932 meeting was given Donald McEachern, M D, Johns Hopkins Hospital, Baltimore, Md, "A Consideration of the Mechanism of Hyperthyroidism Based Upon Its Effect Upon Cardiac and Skeletal Muscle."

Honorable mentions were awarded A B Gutman, M D, Presbyterian Hospital, New York City, "The Effect of Administration of Iodine on the Total Iodine, Inorganic Iodine and Thyroxine Content of the Pathological Thyroid Gland", Lieut Col H Stott, M R C P, I M S, Dean Faculty of Medicine, Lucknow University, Lucknow, India, "The Distribution and Cause of Endemic Goitre in the United Provinces."

Fraternally,  
(Signed) J R YUNG, M D  
Corres Secy

# The Journal of Laboratory and Clinical Medicine

VOL XVIII

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No 5

## CLINICAL AND EXPERIMENTAL

### THYROID GLAND DEFICIENCY IN CHRONIC ARTHRITIS\*

FRANCIS COOLEY HALL, M D, AND ROBERT THORNHILL MONROE, M D,  
BOSTON, MASS

THE complexity of the problems of chronic arthritis is apparent to all, particularly to those who are students of the disease. The American Committee for the Control of Rheumatism defines arthritis as a "generalized disease with joint manifestations." It may involve cartilage, bone, tendon, muscle, and blood vessel, as well as synovial surfaces. It would seem evident that any factor inside or outside the body which may injure directly or indirectly the integrity of a joint must be looked upon as a causal agent. The most successful treatment of the disease is that which seeks to determine the entire etiology in each case, to remove or arrest as many of these factors as may be, and to return the joints, and the patient, to the greatest possible degree of normal function. Such a problem requires the closest cooperation of internists and orthopedic surgeons.

Infection and diet have received much attention in recent years as important etiologic factors. Thyroid deficiency has also been suggested as another, and, while there are many references to it in the literature, its rôle has not been clearly defined. In this paper, we are attempting to show that it occurs quite frequently in patients with arthritis, especially in arthritis of the hypertrophic type, and that its correction leads to improvement in the joint condition.

#### REVIEW OF THE LITERATURE

Endocrine dysfunction has long been suggested as one cause of the greater incidence (3 1) of chronic arthritis among women as compared to men. It is generally recognized that the onset of the disease is often near the menopause, and some writers<sup>1, 2, 3</sup> maintain that menopausal arthritis is more or less distinct from the ordinary hypertrophic (osteoarthritic) or atrophic (rheumatoid) types, whether of infectious or metabolic etiology. There is no agreement as to the gland which is most at fault. The thyroid is incriminated by some, the ovary and pituitary

\*From the Medical Clinic of the Peter Bent Brigham Hospital.  
Received for publication May 27 1932.

by others, and the whole group by still others. Crowe,<sup>1</sup> however, feels that glandular deficiency is not necessary to explain these age and sex differences.

The association of arthritis with myxedema and hypothyroidism has attracted the attention of a few students for many years. Lilwell<sup>2</sup> reports that the incidence of osteoarthritis seems to be abnormally high in regions in England where goiter is endemic, and he quotes McCarrison as saying that in goitrous regions in the Himalaya Mountains the coexistence in the same subject of goiter and arthritis is so common as to suggest a similarity of origin. He relates cases of his own and others from the medical literature, in which arthritis followed closely upon hyperthyroidism which had been treated by x-rays, surgical removal or functional exhaustion of the gland. Nutch in a series of 200 cases of chronic arthritis found that "minor forms of subthyroidism" were very common, while well defined myxedema or goiter of considerable size were seen in 14 per cent of the cases.

Jones<sup>3</sup> writes, "I have frequently observed in women at the menopause, the subjects of osteoarthritis of the knees, a peculiar change in the consistency of the skin and subcutaneous tissues, more particularly of the lower limbs. The overlying skin is usually harsh, dry and not infrequently scaly, has lost its suppleness and is with difficulty lifted up from the subjacent tissues, which to palpitation feel tense, elastic, and, like the skin itself, much thickened. No pitting is to be observed on pressure, and the physical signs appear independently of renal and cardiac complications. The administration of thyroid substance in some instances was quickly followed by a diminution in swelling when present, and also in restoring a more natural consistency to the affected tissues.

"Whether the infiltration of the skin and subcutaneous tissues is identical with the diffuse, brawny infiltration noted occasionally by Girrod in the fingers of patients affected with multiple osteoarthritis of the hands, I am unable to say. As to its nature, one can only speculate, but objectively, at all events, it appears indistinguishable from the solid swelling associated with myxedema. Its localized distribution forbids our identifying it with this affection, but, at the same time, we must remember that physicians following Hertoghe are learning to postulate the existence of states intermediate between swollen myxedema and the normal 'forms frustes,' so to speak, analogous to the larval forms of Graves' disease, and presumably due to variable degrees of thyroid inadequacy."

Other writers confirm Jones as to the frequency of the symptoms of hypothyroidism in arthritic patients. Thus, Emerson comments on the ease of fatigue, paresthesias, parchment like skin, and dry, rough and brittle finger nails. Coates<sup>4</sup> emphasizes the loss of hair, increased weight of the body, dryness of the skin, inability to sweat, scanty or absent menstrual periods and increased sensibility to cold.

McCarrison<sup>5</sup> states that in patients with thyroid gland deficiency "The cartilages are also infiltrated and the joints are painful, stiff and may crackle. 'If the hand is applied to the joint (knee) when flexed, there will be a sensation like the cracking of frosted snow. This is a very characteristic sign, indeed, it is almost pathognomonic.' (Hertoghe) 'Rheumatic' pains and soreness in the bones and joints are a frequent source of complaint, and true rheumatoid arthritis is not uncommonly associated with the disease. Hydrarthrosis may occur (Dulche)."

Cecil and Archer<sup>6</sup> also reported crepitation in the knees with little limitation in motion, as well as excess fat about the hips and breast, round shoulders, lordosis, ptosis and flat feet.

Foster<sup>7</sup> writing about myxedema, says "In adults there is often soreness of the joints and relaxation of the ligaments, leading to flat foot and knock knee. A peculiar grating of the joints on motion has been described. Pains in the back and shoulders are common, and women complain of pain in their arms when doing up their hair, muscular cramps are usual. The muscles, as a rule, fatigue very readily. When standing, the myxedematous patient 'droops all over,' the shoulders fall forward, the head hangs and the body is not erect. Ptosis of the viscera has been attributed to the relaxed abdominal wall."

In their study of 45 cases of hypothyroidism at the Massachusetts General Hospital, Means and Krantz<sup>8</sup> found that all complained of a feeling of fatigue or weakness and of pale and dry skin, 90 per cent had edema of the eyelids and face, increased weight, sensitivity to cold and absence of sweating, 80 per cent had numbness and tingling and loss of hair, and about 70 per cent had brittle nails, 18.9 per cent had symptoms and signs referable to the elbow joints, 56.4 per cent to the knees, and 34.2 per cent to other joints.

Studies of the basal metabolic rate in patients with chronic arthritis have given confusing results. Pemberton and Tompkins<sup>11</sup> found that 80 per cent of their 29 cases fell within normal limits (plus and minus 10 per cent) and that 20 per cent were slightly low (minus 11 per cent to minus 21 per cent). The low rates seemed to bear no relation to the severity of the disease, age or condition of the patient, atrophy of muscles, edema, or other factors. Cecil, Barr and DuBois<sup>12</sup> studied 4 cases of chronic arthritis in the calorimeter and concluded that they had no disturbance of metabolism. Holmes<sup>13</sup> reported normal readings in his 70 cases.

Boothby and Sandiford<sup>14</sup> found basal metabolic readings between minus 15 and plus 15 in 93 per cent of 69 arthritic patients. The rate was below minus 15 in 4 of these patients. Hench<sup>15</sup> found that 80 per cent of 115 arthritic patients had basal metabolic determinations between plus 10 and minus 10, about equally divided on the minus and plus sides. Five slightly higher rates were obtained in one case of thyroiditis and in four cases of thyroid adenomas. In the infectious group (atrophic) six rates were between minus 10 and minus 20. There was a slight tendency for lower normal rates in the senile (hypertrophic) group. He quotes DuBois as having stated that in chronic infectious arthritis it would seem that the change in basal metabolism is such as might be expected in a crippling disease where the patients are forced to lead sedentary lives.

Swann,<sup>16</sup> however, found that of his series of 312 cases, only 61 per cent fell between the normal limits of plus and minus 10, while 14 per cent were slightly increased (plus 10 to plus 15) and 25 per cent were decreased (minus 10 to minus 30). He found no evidence that hyperthyroidism and hypothyroidism are characteristically associated with arthritis. He believed that "abnormal metabolism with a tendency to a minus rate is characteristic of arthritis, especially in the early years, having a tendency to return to normal as the duration of the disease lengthens."

Swann and Spear<sup>17</sup> agreed with Pemberton that the age of the individual and the duration of the activity of the disease had no apparent effect on the basal metabolic rate. They found that in the chronic infectious type of case there was a tendency toward an increased rate and in the hypertrophic case to a decreased rate.

Brown<sup>18</sup> found that of 25 cases of hypertrophic arthritis in which careful basal metabolic readings were made, 16 gave readings less than minus 10, and of these 10 were less than minus 15 and 5 less than minus 20.

Thompson<sup>19</sup> believes that an endocrine disturbance is one of the etiologic factors in arthritis, but believes no single gland to be responsible. We might quote many other writers who believe thyroid or other glandular deficiency to play a part in the etiology of chronic arthritis.

The effect of the administration of thyroid substance to patients with chronic arthritis likewise is in dispute. Jones,<sup>20</sup> Thompson,<sup>19</sup> Mutch, Swann,<sup>16</sup> Brown<sup>18</sup> and Cecil,<sup>12</sup> among others, advocate its use. Pemberton<sup>11</sup> and other writers are in doubt. Swann says that it "does not raise the rate in four fifths of the cases, but if it is carefully given, beneficial results are obtained in spite of the fact that in doses which can be tolerated it may not change the metabolic rate. The chief improvement is in circulation, muscle tone, weight and vitality."

#### PRESENTATION OF DATA

In view of the indeterminate nature of the situation, it seemed of value to contribute an analysis of our experience. For this purpose, we have studied the records of 300 patients with chronic nontuberculous arthritis for the symptoms and signs of thyroid deficiency, the basal metabolic rates, and the effect of thyroid gland therapy when indicated. No selection of cases was made. The records of 100 private patients were taken at random from the files of the last ten years, and, in the same manner, those of 200 patients seen in the wards and arthritic clinic of the Out Door Department of this Hospital in the last seven years. This method had the disadvantage of including some cases which we had not seen, cases with short observation periods and possibly incomplete data from the standpoint of moderate endocrine disturbances. It had the advantage of

representing a true cross section of the sort of arthritic patient we have been dealing with during this period, and of avoiding overstatement

It happened that by chance 150 of the cases fell into the group of atrophic (rheumatoid) arthritis (106 of the hospital and clinic cases and 44 of the private) and an equal number into that of hypertrophic (osteoarthritic) arthritis (94 of the hospital cases and 56 of the private) To avoid confusion, no further subdivisions were made The diagnosis in each instance was arrived at through the gross appearance of the joints and the roentgenographic findings This was often a difficult matter Some of the hypertrophic patients seemed to react like those with atrophic changes Many of those who had had atrophic arthritis for a long time had acquired hypertrophic elements through mechanical irritation and efforts at repair When a case was found to be classified as a mixed type, it was placed arbitrarily in the group to which it most nearly corresponded

This group of patients does not represent a cross section of all types of arthritis Cases clearly infectious in origin, such as gonorrheal arthritis, pneumococcal arthritis, or arthritis in association with rheumatic fever, did not usually reach our clinic, and were eliminated from the cases chosen from the files Those patients whose arthritis was not obviously related to infection, or who had failed to improve with the removal of infection and with other therapy, were the patients generally sent to us They rarely had fever, leucocytosis, or hot joints Readers who see a larger proportion of cases of these types should, therefore, have in mind the character of this group However, we believe some of these patients, especially those with atrophic arthritis, harbored an infectious process which played an important etiologic rôle

Female patients outnumbered the males, as always, in the ratio of about 3 to 1 There were 106 females in the atrophic group and 112 in the hypertrophic, 44 males were classed as atrophic and 38 as hypertrophic The average age of those with atrophic arthritis was 45, and of those with hypertrophic arthritis 54 That this disparity is not as great as usual is due to the fact that many of the patients came to us years after the beginning of their disease

TABLE I

THE INCIDENCE OF SYMPTOMS WHICH MIGHT BE ATTRIBUTABLE TO THYROID DEFICIENCY IN 300 CASES OF CHRONIC ARTHRITIS

		ATROPHIC ARTHRITIS 150 CASES		HYPERTROPHIC ARTHRITIS 150 CASES	
		NO	%	NO	%
1	Sensitivity to cold	64	42.6	66	44
2	Cold hands and feet	73	48.6	76	50
3	Dry skin	59	39.3	94	62.6
4	Ease of tire	135	90	141	94
5	Symptoms out of proportion to physical findings	24	16	38	25.3
6	Arthralgia	24	16	40	26.6
7	Numbness	38	25.3	73	49
8	Brittle nails	38	25.3	66	44
9	Falling hair	23	15.3	59	39.3
10	Edema about the eyes	36	24	58	38.6
11	Obesity	53	35.3	99	66



Table I summarizes the frequency with which occurred the symptoms which might be caused by thyroid deficiency in these patients. Only the positive findings were recorded, where the symptoms were not mentioned, as often happened, they were considered to be absent. Their incidence tended to be greater in the private cases, since they were closely observed in this regard, but the figures for the hospital ward and the clinic cases show the same differences between the two types of arthritis. Patients of both types were equally sensitive to cold (42 per cent of the atrophic cases and 44 per cent of the hypertrophic cases) in that they required more clothing and bedding than other members of their families. Some had always suffered in cold weather, others only since the onset of the arthritis. It is our belief that this symptom is often simply a reaction to illness and does not by itself suggest a glandular disorder.

Those who complained of cold hands and feet were also equally distributed as to type, 48 per cent of the atrophic patients and 50 per cent of the hypertrophic patients. However, this was accompanied in most of the atrophic patients by vasomotor changes such as mottling and swelling, a feature rarely found in the other group. Loss of the normal amount of moisture on the skin, in fact, was found in the majority of the hypertrophic patients. This dryness had to be distinguished from that found with senile atrophy and from the changes due to exposure to cold weather. It involved the hands particularly, but the arms, legs, and feet by no means escaped. The skin was frequently parchment-like, relatively inelastic, and often firmly attached to the flesh beneath. Some patients with atrophic changes of long duration had such skin, but the active cases of this type had characteristically a moist skin.

Nearly all of the patients complained of ease of fatigue. A sense of exhaustion would follow quickly upon even ordinary effort. They would be very tired on rising in the morning, and gather themselves together only slowly as the day wore on. Persistent effort was necessary to keep up with the demands upon them. In the atrophic group this was commensurate with the degree of arthritis and dated from its onset. In the hypertrophic group the striking fact was that these people appeared to be for the most part well nourished and of good physical capacity. Some had carried successfully more than their share of life's burdens. To feel unable to work at the usual speed or for the usual length of time was quite a new experience to them and this was out of all proportion to the extent of the joint involvement, others had been easily tired for years before their arthritis began, but had driven themselves to carry on with an average job. Symptom 5 in Table I illustrates this further. In general, it appeared that the patient with atrophic arthritis felt fairly well apart from his joint pain, unless he was wasted from repeated exacerbations over a long period of time. Certain patients with hypertrophic arthritis, however, had less disability from joint pain, and, though appearing well, would complain of being below par. Others in this group, besides being below par and complaining of ease of fatigue had headaches, and pains referred to muscles and bones as well as joints, with very few objective signs of hypertrophic arthritis. We have considered them under the heading of "Arthralgia."

Paresthesias of the extremities were reported by half of the hypertrophic patients and one-fourth of the atrophic patients, being noted as numbness,

tingling, burning, or prickling. They were sometimes more pronounced in the involved fingers but not limited to them or the hand. Factors which tended to decrease circulation such as exposure to cold tended to produce them. They were therefore, most common after exposure to cold and on waking from sleep, and tended to improve, along with characteristic morning stiffness, as the circulation became more vigorous. They were less commonly met with in the patients with very active atrophic arthritis, occurring more frequently in those of that type with long standing changes.

In one-fourth of the atrophic patients the finger nails were described as ridged, broken and brittle, and in a few there was a tendency for the nail to be excessively dry and to fall out. These symptoms, while twice as common in the hypertrophic individuals were reported in less than half. The greater average age of this group would account for some of the difference, but in certain cases the findings were so striking and the onset so closely linked with the rheumatic process that we were inclined to feel that both could be attributed to hypothyroidism. It is our impression that the incidence of these signs will increase with closer observation.

Edema about the eyes exhibited the same group variation, (24 per cent of the atrophic cases and 38.6 per cent of the hypertrophic cases). Such eyes were those with an appearance of puffiness or pouchiness and congestion about the lids and narrowing of the palpebral fissure. No factor of cardiac or renal disease was present to account for it.

Subcutaneous edema elsewhere than about the eyes, was noted very infrequently and about equally in the two groups. It was observed in the skin of the ankles, hands (dorsum) and face especially. It was only occasionally due to heart disease with congestive failure. Localized waxy areas of myxedema and fat pads over the shoulders as mentioned by some writers in the literature quoted above, were rarely seen.

Thirty-five per cent of the patients with atrophic arthritis were definitely overweight, that is, more than 15 per cent over their ideal weight, while 66 per cent of the hypertrophic patients were obese. This is further evidence suggestive of a different constitutional background of health and interaction with disease. The typical rheumatoid patient is wasted and eats listlessly of a restricted diet. The hypertrophic person is usually robust or buxom and blessed with a generous appetite. As pointed out elsewhere (Monroe and Hall<sup>15</sup>) starch indigestion is more common to him also.

Enlargement of the thyroid gland was reported as having been present in 4 cases in each group at some time. One in each group had been subjected to subtotal thyroidectomy for hyperthyroidism in the past, in one hypertrophic arthritis followed directly. Three had adenomas and three had colloid goiters. None had increased metabolic rates (over plus 10) at the time of this investigation.

Thirty-six per cent of the women with atrophic arthritis dated the onset of their disease at the menopause or a year before or after it. This was true of the majority of those women in the hypertrophic group, where 64 (57 per cent) so reported.

*Basal Metabolic Rates*—There were 206 determinations of the basal meta-

boke rate made on 106 patients with atrophic arthritis, and 216 were made on 108 patients with hypertrophic arthritis. No tests were done on 44 of the first group, 6 of which were private patients, and none on 42 of the second group, 5 of which were private patients. The test was almost routine in office practice because of the author's interest in this problem. In some of the clinic cases it was omitted because of the difficulties encountered in such an ambulatory group. In the hospital wards it was done chiefly when there was reason to suspect an abnormal result. No case was excluded for lack of this test. The ward and clinic cases were done in the hospital metabolism laboratory by a technician and an assistant resident in medicine. The Benedict-Roth apparatus was used. The private cases were done by a trained worker using the same apparatus. It was unusually difficult to secure satisfactory curves and checks on tests done on these patients. This was due not to temperature or pulse elevation but to inability to secure good relaxation. Many people came from a distance and had to be allowed to rest for an hour or more before they could breathe quietly. Pain, discomfort, and nervous tension were sometimes great enough to invalidate the test or cause it to be given up. Repeated tests usually showed lower rates, when these factors were overcome. The errors are all on the side of increased oxygen consumption. Better results might be obtained under improved conditions and should show fewer readings over plus 10 per cent. Table II shows the results, with percentages on each bracket.

TABLE II

THE LOWEST BASAL METABOLIC RATES IN 106 CASES OF ATROPHIC ARTHRITIS (206 TESTS) AND 108 CASES OF HYPERTROPHIC ARTHRITIS (216 TESTS)

B M R	ATROPHIC ARTHRITIS		HYPERTROPHIC ARTHRITIS	
	NO CASES	%	NO CASES	%
+26 to +29	2	1.8	0	0
+21 to +25	2	1.8	1	0.9
+16 to +20	6	5.6	1	0.9
+11 to +15	2	1.8	5	4.5
+ 1 to +10	18	17.0	16	14.8
0 to -10	38	36	31	28.7
-11 to -15	19	17.9	17	15.7
-16 to -20	15	14.1	20	18.5
-21 to -25	3	2.7	10	9.3
-26 to -29	1	0.9	7	6.4
Totals	106	99.6	108	99.7

It is at once apparent that the majority of cases fell within normal limits. Between rates of plus 10 and minus 10 there are 53 per cent of the atrophic group and 43.5 per cent of the hypertrophic group, between plus and minus 15 there are, respectively, 72.7 per cent and 63.7 per cent. In the abnormal ranges, the differences are rather striking. Of the atrophic cases, 9.2 per cent had rates above plus 16 as compared with 1.8 per cent of the hypertrophic cases. Below minus 16 are found 17.7 per cent of the atrophic cases, and 34.2 per cent of the hypertrophic cases, while below minus 20 there are only 3.6 per cent of the atrophic cases and 15.7 per cent of the hypertrophic cases. There is, thus, a

definite tendency for the hypertrophic patient to have a metabolic rate below normal. Hypometabolism is found in the other type, but to a much smaller extent. This is brought out in Chart 1.

*Results of Thyroid Gland Administration*—No simple experiment, in which no other treatment than thyroid gland substance was given, was possible to carry out in our series of cases, except to a certain extent in a few instances. While presentation of such a group of cases affords clear proof of the utility of this medication, as will be shown in a subsequent paper, they are few in number and are not entirely similar to the average case of arthritis with which one is confronted. We were forced to treat the patient as a whole. In general, this consisted in prolonged bed rest at home or in a hospital, a diet rich in vitamins, usually with cod liver oil, viosterol, yeast, orange or tomato juice added, attempts to correct postural and mechanical defects, the search for and care of infections, and attention to concomitant diseases, as necessary. Obviously, the question as to which element in such a manifold program was responsible for any subsequent improvement in the individual patient was often

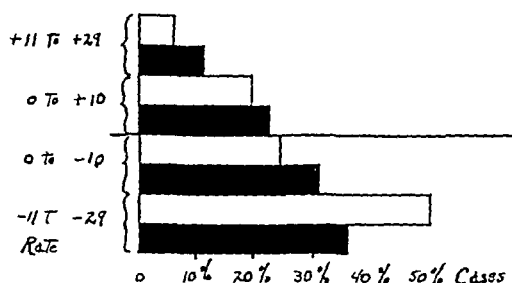


Chart 1—The lowest basal metabolic rate determination in 107 cases (206 tests) of atrophic arthritis (shaded areas) and 108 cases (216 tests) of hypertrophic arthritis (unshaded areas). While about half of the cases lie between  $\pm 10$  there is a definite tendency for the rate to be low in the rest, particularly in the hypertrophic type.

extremely difficult to answer. The rôle played by thyroid medication was settled separately for each case by both authors, one of whom, having had little association with many of the patients, acted to a degree as a neutral observer.

Thyroid extract was not given routinely. It was withheld from 5 atrophic arthritic patients and 2 hypertrophic arthritic patients in the private group, and from 42 atrophic and 32 hypertrophic patients in the ward and clinic group. This means that therapeutic tests were almost universal in one group, but not in the other. Harmful effects were limited to sensations of dizziness, nervousness, tension, headache, and insomnia. They promptly disappeared when the thyroid extract was omitted and serious symptoms were not encountered. This may have been due to our policy of giving small initial doses (one quarter grain desiccated thyroid once or twice daily).

It was decided that thyroid extract had no effect when there was no demonstrable improvement following its use. It was described as of "temporary value" when it seemed to accelerate improvement for a short time, but was without consistent effect throughout the period of observation. Its effect was "good" when it was of unquestioned value in our and the patient's estimation over and above all other elements of treatment for a considerable period of time.

These beneficial effects require some description. Objectively, there was a change from a dry to a moist skin, and an increase in pliability and wrinkling where it had been abnormally firm and thick. Congestion about the eyes and throat improved. Those who were obese lost weight, particularly about the abdomen, hips, and ankles. The affected joints were usually more normal in size due to a loss of boggy swelling, then function was measurably improved. The subjective benefits were often out of proportion to the immediate improvement in physical signs. The patient felt better at once and was better organized nervously, so that he bore his infirmities with great equanimity. Joint sensitivity abated quickly. Physical activity without pain was increased even before there was any demonstrable change in the joints that we could measure. There was less sensitivity to cold and less trouble with cold hands and feet.

Table III shows the results of thyroid therapy in this series of cases. No effect was observed in 71.8 per cent of the atrophic group (46 per cent of the private cases and 87.5 per cent of the hospital cases), and in 31.0 per cent of the

TABLE III

THE EFFECT OF ADMINISTRATION OF THYROID EXTRACT TO 103 PATIENTS WITH ATROPHIC ARTHRITIS AND 116 PATIENTS WITH HYPERTROPHIC ARTHRITIS

RESULT	ATROPHIC ARTHRITIS				HYPERTROPHIC ARTHRITIS			
	PRIVATE CASES		HOSPITAL CASES		PRIVATE CASES		HOSPITAL CASES	
	NO	%	NO	%	NO	%	NO	%
No effect	18	46.1	56	87.5	13	24.1	23	37
Temporary good effect	8	20.5	4	6.3	5	9.2	18	29
Good effect	13	33.3	4	6.3	36	66.7	21	34
Totals	39		64		54		62	

hypertrophic group (24.1 per cent of the private cases and 37 per cent of the hospital cases). Beneficial effects, both temporary and good, were obtained in 28.2 per cent of the atrophic group and in 69 per cent of the hypertrophic group.

The difference in value of the drug in the hospital cases as compared with the private cases can be explained in several ways. In the latter group the patients were observed for a longer period of time by one group of physicians particularly interested in arthritis, and on the watch for the suggestive signs and symptoms of glandular deficiency pointing to a need for a basal metabolic rate determination. In the former group, the patients were seen for shorter periods of time by a varied group of internists, they had more complicating factors of disease and were less able to follow strict treatment.

It is evident that thyroid was of real value in one-third of the private cases of atrophic arthritis and in only one sixteenth of the ward and clinic cases. In the hypertrophic cases there were definitely fewer failures, more temporary effects, and many more good effects. Where good effects were obtained, they appeared soon after the drug was given and disappeared soon after it was stopped, it seemed to meet a real deficiency. On the other hand, the temporary good effects seemed more explicable on grounds of stimulation or other action

of the drug, as improvement was accelerated by its use but continued after its withdrawal. In this group, too, care in the application of other factors of treatment had much to do with the way thyroid acted. Temporary value was noted more in the cases which could take adequate rest and diet. In other cases, thyroid seemed to be of no help, and sometimes caused an increase in symptoms. Patients with active or chronic infections were rarely able to take it.

As to the effect of the administration of thyroid on the basal metabolic rates of this group of patients, our data are rather inconclusive. Many patients had only one test performed because they were not studied intensively from this standpoint (as in the private hospital cases) or because of the difficulty in getting outside patients back for the test and the limited facilities of the metabolism laboratory. In those who had more than one test there was a tendency to omit continued observation when improvement set in for the same reasons. Demonstration of the fact that arthritic patients with definitely low metabolic rates responded to thyroid therapy, with a uniform return of the rates to normal, would be valuable evidence that hypothyroidism is present, for it is known that the metabolic rate is quite sensitive to thyroid when it is needed, but that large doses will not affect it when it is not needed.

Of 56 cases of hypertrophic arthritis that had two or more metabolic rate determinations 25 (44 per cent) showed a rise of the rate to normal, from an average initial rate of minus 13 to an average final rate of plus 0.9. Twenty-five (44 per cent) showed no definite change from the first on subsequent examinations although the figures are rather unsatisfactory, and 6 (11 per cent) showed later a lower rate than at first. The fallacy of excluding hypothyroidism on the basis of one normal rate determination is shown by the fact that 13 whose first test showed readings from plus 6 to minus 11 later showed lower rates (minus 17 to minus 28) and still later normal readings (plus 10 to minus 11) following thyroid therapy.

Of 60 cases of atrophic arthritis that had repeated metabolic rate determinations, 16 (26 per cent) showed a rise of the rate to normal, from an average initial rate of minus 14 to an average final rate of plus 4. Twenty-nine (48 per cent) showed no definite change, and 15 (25 per cent) showed lower final rates than the initial ones. Thus the figures in this group are less striking than in the hypertrophic group and in agreement with other differences before noted.

Data from a few closely followed cases with the stigmata of hypothyroidism and low initial metabolic rates, which show a loss of symptoms and normal rates after thyroid therapy, have convinced us that if it had been possible to have more metabolic studies in comparable cases the results would have been similar in a high percentage. The cases which showed no change in metabolism after thyroid therapy were for the most part those which lacked more of the stigmata of hypothyroidism. Some of the latter improved on thyroid therapy, probably due to the non-specific effects of the drug while others did not respond at all.

Further evidence of the value of thyroid therapy is presented in Charts 2 to 5 in which the general results of treatment have been plotted. This assessment was arrived at by grading each case on a basis of one to four from two standpoints, the extent of joint involvement and the amount of pain and disability, after the practice of the Mayo Clinic. The number of diseased joints was estimated by physical

examination, since x-ray films of the whole skeleton were not feasible. A person with one knee or a few fingers alone involved was rated as one, with both hands and fingers, wrists and a few other joints (perhaps one half of the total joints of the body), as two, and with apparently all joints, as four. Pain of a mild sort, not

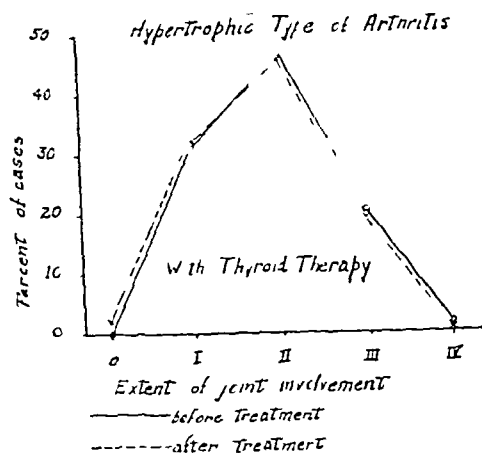


Chart 2-A.

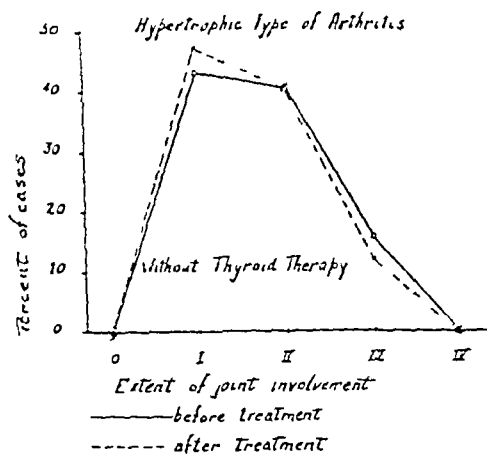


Chart 2-B

Chart 2-A—The extent of joint involvement in 118 cases of hypertrophic arthritis before and after treatment which included thyroid substance. For explanation of degree of extent of involvement see text.

Chart 2-B—The extent of joint involvement in 32 cases of hypertrophic arthritis before and after treatment which did not include thyroid substance. For explanation of degrees of extent of involvement see text.

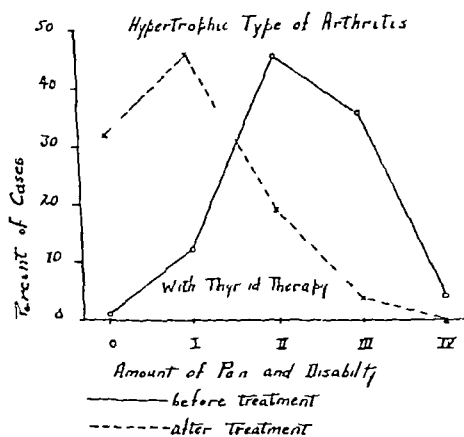


Chart 3-A

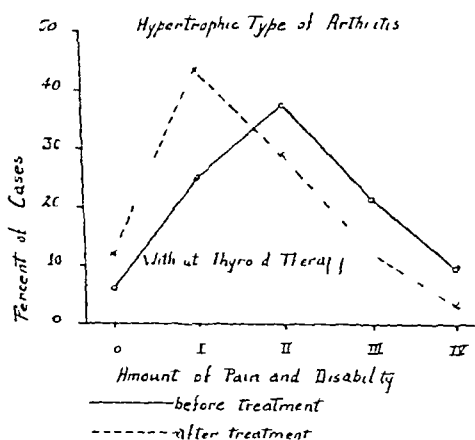


Chart 3-B

Chart 3-A—The amount of pain and disability in 118 cases of hypertrophic arthritis before and after treatment in which thyroid was included. For explanation of the rating of pain and disability see text.

Chart 3-B—The amount of pain and disability in 32 cases of hypertrophic arthritis before and after treatment in which thyroid was not included. For rating of amount of pain and disability see text.

causing limitation of activity, was estimated as one, pain and disability enough to cause moderate restriction, two, complete prostration and helplessness, four. The hospital and clinic cases were followed for an average of one year and three months, the extremes being one month and seven years. The private cases were followed for an average of two years. Patients treated with thyroid were

grouped in one series of charts, to contrast them with those who did not have thyroid

Charts 2-A and 2-B show that in the hypertrophic cases the extent of involvement did not change as a result of treatment. No joints with definite degenerative,

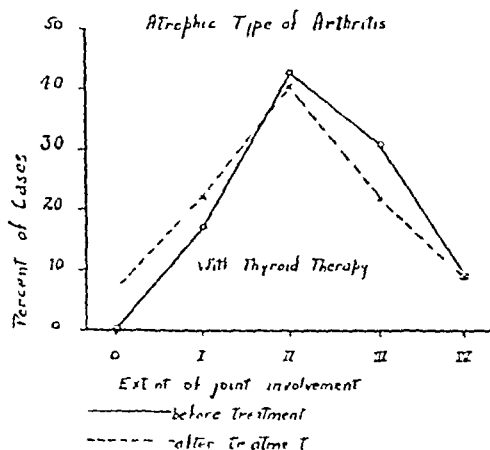


Chart 4-A

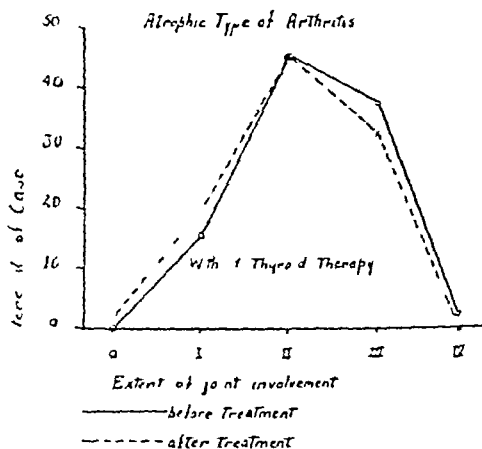


Chart 4-B

Chart 4-A—The extent of joint involvement in 106 cases of atrophic arthritis before and after treatment in which thyroid was included. For explanation of degrees of extent of involvement see text.

Chart 4-B—The extent of joint involvement in 46 cases of atrophic arthritis before and after treatment which did not include thyroid. For explanation of degrees of extent of involvement see text.

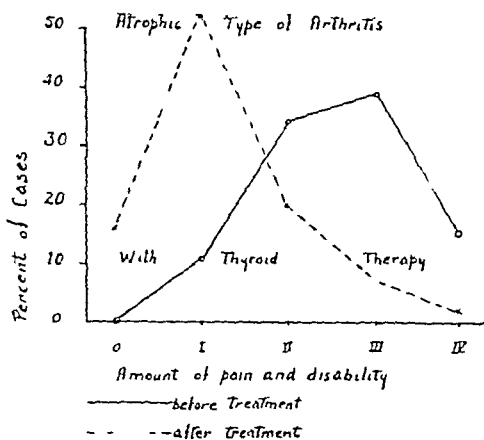


Chart 5-A

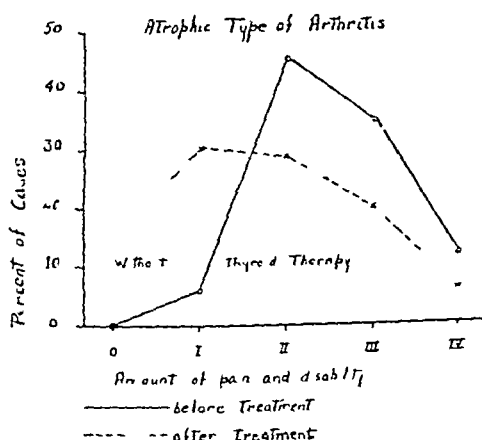


Chart 5-B

Chart 5-A—The amount of pain and disability in 104 cases of atrophic arthritis before and after treatment which included thyroid. For rating of amount of pain and disability see text.

Chart 5-B—The amount of pain and disability in 46 cases of atrophic arthritis before and after treatment which did not include thyroid. For rating of the amount of pain and disability see text.

osteoarthritic lesions were restored to normal, of course. On the other hand, the close approximation of the two lines before and after treatment shows that, with or without thyroid, the disease appeared to be arrested, at least for the duration of this period of observation. The similarity of the two groups of cases with regard to severity is also to be seen at a glance. Those who received thyroid were rated as



being mostly "one" and "two" in extent of involvement, and so were those who did not receive thyroid

Charts 3-A and 3-B show that pain and disability were considerably decreased after treatment in the hypertrophic group, and without the use of analgesics. Those who did not receive thyroid (Chart 3-B) were rated as mostly "one" to "three," and "zero" to "two" later. However, the separation of the lines before and after treatment is much more striking in the group which took thyroid, they were for the most part "two" and "three" before, a bit more severe than the other group, and became mostly "zero" to "one" afterwards. There were many patients who secured complete release from pain, and while joint motion in some others continued to be limited, functional activity was often extraordinarily increased. Very few patients in this group continued to have extreme disability.

Charts 4-A and 4-B show, in the atrophic arthritic patients, the same tendency for the degree of involvement to be fixed, though there is a suggestion that some restoration toward normal has taken place. There is more separation of the lines and shift to the left here than in Charts 2-A and 2-B. The general onset of the disease process is also apparent as before.

Charts 5-A and 5-B show a more favorable balance for the thyroid treated atrophic cases as against the nonthyroid treated cases than one might expect from Table III. While there is considerable improvement apparent without thyroid, most of the cases remain in grades "one," "two," and "three" of disability and pain. Very few cases are left in grade "three" where thyroid was given, and many more have reached grade "one."

#### DISCUSSION

This analysis of 300 cases of chronic arthritis suggests that some degree of thyroid gland deficiency may be a common contributing factor, since the symptoms and physical signs usually attributed to such deficiency were found in a large number, since there was a definite tendency for the basal metabolic rate to be low, and since it seemed clear by all tests that thyroid gland substance added to the general treatment was of value in many. The evidence was consistently more impressive for the hypertrophic type of arthritis than for the atrophic type. Hypothyroidism is, therefore, worth searching for and treating in this disease.

Each of the symptoms and signs considered in the literature to be typical of thyroid gland deficiency, and listed by us in our series of patients are found individually, of course, in a variety of diseased states and can be caused by various factors. But the frequent occurrence of several of them together, as shown in Table I, creates an impression that a true lack of thyroid secretion is responsible. They are much more frequent in the hypertrophic type of arthritis than in the atrophic type, and it is in that type that the metabolism rates were most frequently subnormal and that the glandular medication proved to be of more consistent and permanent value. As has been shown, one who develops hypertrophic arthritis is quite a different type of individual from the one who develops atrophic arthritis, and the subsequent improvement in his general condition and in his joints is definite, often striking, and frequently out of all proportion to what one would expect from the correction of such moderate deficiencies.

We have noted frequently, although we have no figures to offer, an appearance of congestion and edema about the tonsils and pharynx and the uvula in these patients, particularly those with hypertrophic arthritis and evidence of hypothyroidism. The eyelids often share in this process. It seems quite probable that it represents an infiltration due to thyroid gland deficiency, as noted by Foster.<sup>9</sup> The redness of the anterior pillars and enlargement of the tonsils has often led to their removal as a focus of infection, usually without benefit to the patient. We have seen the same congestion of the throat in patients who have had adequate tonsillectomy.

The difficulties in securing valid basal metabolic rate determinations in our patients, particularly in the large ambulatory group, has been mentioned. The arthritic individual is typically one who cannot understand that he has a general disease, but feels that he is well except for his arthralgias. Consequently, he does not give up to it, and drives himself to keep going from fear of disability and the accusation of being a neurotic. This nervous tension is reflected in the irregularity of the kymograph tracings when he submits to a metabolism test. Pain also prevents proper relaxation as well as activity before the test. If the patient comes to the laboratory from any distance, he requires more time to rest than other types of patients. A normal metabolism report in an arthritic patient, therefore, particularly if it be the first test, cannot be taken as a true reading or as excluding hypometabolism. The error is always in the plus direction, not in the minus one. In many patients with a moderate degree of arthritis there is a marked fall in the reported metabolic rate, often 20 per cent after a few days' rest and release from pain. Swaim has emphasized this also. We feel sure that our tabulated results of the metabolism determinations in this series of patients err in being in general too high. If all the patients could have been tested only after a period of hospitalization, perhaps more would have been found to be subnormal, as was the case in our hospitalized private patients.

Critical examination of metabolism studies has led many writers to distrust the metabolism rate as an infallible index of the activity of the thyroid gland. Benedict<sup>20</sup> has shown that body weight and the state of nutrition influence the rate importantly. A depleted, underweight individual may have a minus 20 reading, and an obese person a plus 20 reading. Exhaustion may cause a low reading, and recovery from acute infections may be attended by low readings, as shown by Gordon and Rabinowitch.<sup>21</sup> Several have noted that a person whose habitual level is minus 10 may have hyperthyroidism with a rate of only plus 15 or plus 20. Similarly, it is probable that one whose usual level is plus 10 may have hypothyroidism or myxedema with readings of only minus 10 or minus 15. It seems fair to say that the metabolic rate is nonspecific and only one sign of thyroid activity, to be considered with the clinical signs and symptoms pointing to hyperactivity and hypoinactivity. Recognition of this fact may serve to place hypothyroidism, as distinct from myxedema, on a more clearly established basis than it has enjoyed in the past.

The mode of action of thyroid gland extract in relieving the general and local condition in patients with chronic arthritis may also be both specific and nonspecific. The benefit in any particular case from the use of thyroid therapy is, of course, no proof that hypothyroidism or myxedema are present. Adrenalin has a

wide range of usefulness apart from adrenaal insufficiency. Similarly, thyroid extract aids in reducing weight, increases the circulation and is concerned with calcium metabolism. We are convinced that it meets and controls a real deficiency in certain cases of arthritis, as our figures show. We cannot prove that it may not act in some of these cases as a nonspecific agent, however.

If myxedematous infiltration of the joint tissues occurs, it should act to clear up such collections as it does those in other body tissues, such as the heart, kidney, peritoneal cavity, eye, and skin. The senior author has had several cases of mild arthritis with joint swellings which disappeared after thyroid extract was given, and other patients with arthralgias and symptoms of deficiency, but no joint change, who were relieved in a similar fashion.

This is the most direct evidence which we have had that the drug can act locally on the diseased joints, and substantiates the clinical observations which we have quoted above from the literature. We have been unable to find any mention of pathologic examination of the joint structures in myxedematous patients. Autopsies are rarely done in this condition and the joints are usually spared. That they should escape so generalized a process seems improbable. The bony changes in cretinism, however, have been well described and seem to be due to poor circulation and hence to poor local nutrition.

Poor nutrition of the joints has long been considered to be a factor in hypertrophic arthritis. Nichols and Richardson named this type "degenerative," while others use the term "senile." Definite local causes of poor circulation have been described in the form of arteriosclerosis and endarteritis. They apply also to myxedema<sup>9-10</sup> in which arteriosclerosis occurs prematurely and there is slowing of the rate of blood flow out of proportion to the metabolic rate, retardation of all tissue growth, and often a secondary anemia. It is quite possible that a moderate decrease of nutrition to the joints from any cause may be of more importance than a similar condition in other tissues, due to the trauma to which joints are subjected. Those which are particularly exposed to mechanical stress and strain, the terminal joints of the fingers, the spine, hips, and knees, are the joints of predilection in hypertrophic arthritis. Long continued mild thyroid deficiency may be comparable to long standing dietary deficiencies in causing generalized tissue change. Therefore, it may be that thyroid therapy can act even in the absence of myxedematous joint infiltrations in mild cases of thyroid deficiency and in cases without deficiency by improving the circulation and nutrition.

Thyroid extract also is concerned with the regulation of calcium and phosphorus metabolism. The myxedematous patient has diminished rates of exchange of these substances. This may amount to as much as 40 per cent retention of calcium, as shown by Aub and Bauer.<sup>21</sup> Roentgenograms of his hands show dense bone, while roentgenograms of the hands of hyperthyroid patients show the bone thinner than normal. That the hypertrophic patient is one who shows abnormal deposition of calcium in bones, joints, ligaments, and blood vessels seems more than coincidence, and more than can be explained on the basis of age. Moreover, Pemberton<sup>22</sup> has shown by experiments on dogs, that reduction of the blood supply to the patella leads to abnormal calcification. Thyroid therapy might be expected to bring about a normal exchange of calcium and phosphorus in these arthritic patients.

Care must be exercised in the selection of patients who are to take thyroid therapy. Routine use of it is irrational and must lead to disappointment and even harm, particularly where there is an element of cardiopathy. It is rarely indicated in active cases of chronic atrophic arthritis of short duration. In this group the etiologic factors are more clearly infection, depletion, exhaustion, and dietary deficiencies, the outstanding stigmas of thyroid deficiency are usually lacking, and low metabolic rates here are due to these causes and not thyroid deficiency. In such patients, and in excessive doses in any patient, it may act as a toxic substance and increase joint pain, and should be used with greater caution. Its greatest usefulness is in those arthritic patients who have signs and symptoms of thyroid deficiency, these include the majority of the hypertrophic type and a small number of the atrophic type of long duration and 'mixed' or hypertrophic elements.

We have found it wise to accustom ourselves to the use of one brand of thyroid gland preparation only, since the products on the market vary widely in potency. Reid Hunt<sup>23</sup> has shown that this may amount to as much as 2500 per cent. It should also be noted that 'fresh' gland substance may be only one-fourth or one-half as potent as the desiccated substance. Textbooks advocate as a small dose one gram of the desiccated gland substance three times a day. This has proved quite excessive in our experience. To begin with, such amounts often lead to toxic manifestations and to fear of the drug on the part of the patient. We, therefore, begin with one-fourth or one-half gram of the desiccated substance daily, and increase it gradually, as the condition of the patient or the metabolic rate suggest.

Toxic manifestations are to be sought for from the start. The first to appear are dizziness and a sense of tension. Then headache is complained of, or a feeling of pressure over the eyes, sleeplessness, emotional instability, malaise or nervousness. Palpitation and tremor have rarely occurred. All these symptoms rapidly disappear when medication is withdrawn. It is important to bear in mind that after a patient has been taking a maximum dose for some weeks, the optimal maintenance dose may become one quarter of this, or even none at all. It is the general belief that a myxedematous patient needs to take thyroid continuously for the rest of his life. Sturgis<sup>24</sup> has had cases which suggest that this may not always be so. However, where need for the drug is temporary, one should question whether one is dealing with a true thyroid deficiency or with a case which responded temporarily to the by effects of the drug. A simple way to avoid overdosage is for the patient to omit thyroid for the first week of every month. This has the further advantage of acting as an indication for dosage. In this way we have had only transient ill effects, and few of them.

The evidence of the effect of thyroid medication is to be found in the response of the metabolic rate and the condition of the patient. As Swaim<sup>25</sup> and as Peters and Van Slyke<sup>26</sup> have noted, the former is not to be followed blindly. Complete clinical recovery may take place in myxedema on small doses of thyroid without a significant rise in the metabolism, and serious discomfort may ensue if the dose is increased in order to make the rate normal. The same is true in arthritis. There is, indeed, often an initial fall of the metabolic rate to a lower level and then a rise toward normal, this occurred in a certain number of our cases which were closely studied. Swaim has observed this tendency for the rate to fall to lower levels under

treatment, but he observed a return to normal later on thyroid alone in only 20 per cent. A return to normal occurred in 44 per cent of our hypertrophic cases and in 26 per cent of our atrophic cases that had two or more metabolic rate determinations, and over a period of time which suggested that the rise was due to the use of thyroid rather than to a response to general treatment.

Clinically, there is first an increased sense of well being. The patient feels the cold less, often eats with better appetite and sleeps more restfully, there is a relief of tension and he makes more of an effort to carry out instructions. Rarely is there immediate improvement in the joint condition. He improves, as most arthritic patients improve, first in his general health and later in his joints, skin and other tissues. Definite increase in function is usually found, as our charts show. This sometimes is more subjective because of lack of pain and stiffness than objective, for there may be little or no increase in the range of motion. This is in keeping with our idea that the most one can do medically in treating this disease is to arrest its progress. We cannot restore badly damaged joints to normal. Reconstruction by orthopedic measures comes later after arrest. Pain may disappear slowly but the disappearance of pain is sometimes one of the striking effects of thyroid therapy.

Those who would advocate the addition of a particular therapy in the already complicated management of the disease, which is often characterized by spontaneous remissions, must meet the objection that any treatment sufficiently prolonged will always meet this natural stage of arrest. Probably 40 to 50 per cent of arthritic patients recover from their acute attack regardless of treatment. It is, therefore, not easy to be convincing in describing the results of thyroid therapy in them. Our object in treatment should be the control of the disease over a period of years. We have followed enough patients for some years to feel that this gland substance is of especial value in preventing recurrent attacks.

Finally, we wish to repeat what we said in the introduction: "The most successful treatment is that which seeks to determine the entire etiology in each case (of arthritis), to remove or arrest as many factors as may be and to return the joints, and the patient, to the greatest possible degree of normal function." These studies lead us to believe that thyroid deficiency is one, but only one, of these factors. It requires correction along with diet, fatigue, infection, and trauma. We also feel that the nonspecific effects of thyroid are valuable enough to warrant its use in certain other cases of arthritis in which glandular deficiency cannot be proved. This is true particularly for the cases associated with vascular disease, chiefly the hypertrophic arthritis cases and a few of the long standing atrophic cases.

#### SUMMARY AND CONCLUSIONS

1. Three hundred patients with chronic, nontuberculous arthritis, have been reviewed for the signs and symptoms of thyroid deficiency, the basal metabolic rates, the action of thyroid gland therapy, and the results of medical treatment, which included thyroid substance. Of these, 150 were of the atrophic type of arthritis and 150 were of the hypertrophic type.

2. In the hypertrophic group of patients

a The symptoms and signs of hypothyroidism occurred with great frequency, with no other obvious explanation for them

b The basal metabolic rates were below minus 10 in 50 per cent, and below minus 15 in 34.2 per cent of 108 patients, even though most of them were determined under inadequate conditions of rest and in the presence of pain. In 42 patients no basal metabolic rate determinations were made

c They were for the most part well nourished individuals and no other explanation for the hypometabolism was apparent except for the glandular deficiency

d Thyroid gland therapy was of permanent beneficial effect in 49.1 per cent of 116 patients in whom it seemed wise to use it

### 3 In the atrophic group of patients

a The symptoms and signs of hypothyroidism were met with less frequently and generally would be accounted for on a basis of depletion and undernutrition

b The basal metabolic rates were below minus 10 in 35.6 per cent and below minus 15 in 17.7 per cent of 106 patients. No basal metabolic rate determinations were made in 44 patients

c Definite glandular deficiency seemed present in only a small number, and there was usually another explanation for the hypometabolism

d Thyroid gland therapy was of permanent beneficial effect in 16.5 per cent of 103 patients in whom it seemed wise to use it

4 Thyroid deficiency appears to be a contributing etiologic factor in certain patients with chronic arthritis, as well as infection, dietary deficiency, trauma, and depletion. Its correction, when present, improves the general well being of the patient, aids in the relief of joint pain and disability, and helps to lay a foundation for permanent control of the arthritis, probably through better joint nutrition

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## CHEMICAL CHANGES IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS\*

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CLOSE similarity in the chemical changes in the blood of dogs in experimental acute peritonitis and high intestinal obstruction has been noted by Haden and Orr.<sup>1, 2</sup> In experimental peritonitis no constant change was noted in the carbon dioxide combining power, whereas, in high intestinal obstruction there was a constant rise. A similar picture was observed in experimental dehydration, except in the chlorides which showed an increase.<sup>3</sup>

Sweet<sup>4</sup> has expressed the belief that the cause of death in high intestinal obstruction and acute pancreatitis is the same. Others have observed that the cause of death resulting from complete external drainage of the pancreatic secretion is dehydration and acidosis produced by the loss of sodium and chloride ions.<sup>5, 6</sup> Elman and Hartmann<sup>7</sup> found that by total loss of the pancreatic juice through external drainage there resulted an uncompensated acidosis. If vomiting occurred, however, the acidosis was less marked or even replaced by an alkalosis due to superimposed loss of gastric juice as a result of the vomiting.

Since there is some similarity between the chemical changes in the blood in acute high intestinal obstruction, acute peritonitis, dehydration and complete loss of pancreatic juice, we were stimulated to study the chemical changes in the blood produced by experimental acute pancreatitis.

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## METHODS

All operations were done under ether anesthesia with aseptic technic. Injections of gall bladder bile, infected gall bladder bile, sodium taurocholate and ether were made either by syringe method, (under minimum pressure) or under 100 millimeters of mercury pressure directly into the major pancreatic duct (ductus Santorini) without traumatizing the pancreatic peritoneum. The duct was then ligated with silk and severed. Animals were kept in metabolism cages and given water ad libitum.

The following standard blood chemical methods were used. Urea nitrogen was estimated according to the Van Slyke and Cullen modification of the Marshall method,<sup>8</sup> nonprotein nitrogen according to the method of Folin and Wu,<sup>9</sup> blood chlorides on the tungstic acid filtrate by the method of Gettler,<sup>10</sup> the carbon-dioxide combining power by the method of Van Slyke and Cullen,<sup>11</sup> sugar according to the Folin and Wu method,<sup>12</sup> and creatinine by the method of Folin and Wu.<sup>13</sup>

## EXPERIMENTAL OBSERVATIONS

Forty-two animals were used in this series of experiments. Six of these animals were discarded because of surgical complications. Detailed chemical studies of fifteen dogs are recorded in Tables I, II, III, IV, V, and VI.

TABLE I

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS PRODUCED BY INJECTION OF GALL BLADDER BILE INTO DUCTUS SANTORINI

DOG NO	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 CC					CO. COMBINING POWER	
			UREA NITROGEN	TOTAL NON PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg	mg	mg	mg	mg	vol per cent	
1	3½ cc bile	0	9.3	22.4	460	101	1.5	35.7	Dog died Pancreatic necrosis
		1	21.0	49.2	470	52	2.2	36.6	
2	5 cc bile	0	10.2	25.0	510	87	1.4	36.6	Dog died Pancreatic necrosis
		1	38.5	62.5	450	53	1.6	40.4	
		2	57.4	88.3	450	52	1.7	44.7	
		3	96.6	143.0	460	124	1.9	44.3	
		4	128.0	167.0	480	133	1.9	44.3	
		6	130.8	273.0	460	112	2.9	47.7	
3	6 cc bile under 100 mm of mercury pressure	0	9.8	28.2	520	98	1.4	38.5	Dog died Gangrenous pancreatitis
		1	14.0	29.4	510	79	1.2	40.4	
		2	14.9	29.7	530	88	1.2	39.5	
		5	15.8	30.3	500	79	1.2	41.4	
		6	46.7	83.3	510	83	1.4	34.7	
		7	35.0	67.5	510	108	1.7	40.4	
		8	39.2	68.3	490	114	1.5	41.4	
		9	44.1	87.3	500	105	-	40.4	
		10	42.0	83.3	480	108	-	-	



**TABLE II**  
**CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS**  
**PRODUCED BY THE INJECTION OF MICROORGANISMS INTO DUCTUS SANCTORINI**

DOG NO	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C C					CO <sub>2</sub> COMBINING POWER	
			UREA NITROGEN	TOTAL NON PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg	mg	mg	mg	mg	vol per cent	
4	15 cc saline suspension B coli	0	14.0	28.5	520	86	1.2	39.5	Dog died Suppurative pancreatitis
		1	49.0	85.3	500	25	1.4	40.4	
5	15 cc saline suspension B coli	0	9.3	23.6	540	82	1.2	39.5	Dog died Pancreasedematous fat necrosis
		1	21.0	46.8	400	85	1.5	37.6	
		2	53.7	90.3	250	93	3.2	36.6	
6	8 cc bile with B coli	0	12.1	23.8	540	83	1.2	33.8	Dog died Purulent pancreatitis
		1	28.0	46.0	530	-	1.4	-	
		2	30.3	50.8	500	69	1.5	40.4	
		4	32.2	60.0	540	50	2.4	45.3	
		10	18.6	28.0	500	30	1.8	57.6	
7	19 cc saline suspension B coli	0	30.3	52.6	450	73	2.6	40.4	Dog alive and apparently healthy 42 days postoperative
		1	51.3	107.2	400	83	3.2	41.4	
		2	81.2	135.0	450	44	4.3	39.5	
		5	46.7	107.0	400	48	4.0	41.4	
		11	42.0	72.3	460	52	2.0	35.7	
		16	32.6	48.2	480	50	2.2	37.6	
		25	31.3	107.2	450	62	2.0	34.7	
		30	37.3	78.0	500	72	1.4	37.6	
		42	36.4	58.8	510	62	2.0	39.5	

**TABLE III**  
**CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS**  
**PRODUCED BY INJECTION OF SODIUM TAUROCHOLATE INTO DUCTUS SANCTORINI**

DOG NO	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 C C					CO. COMBINING POWER	
			UREA NITROGEN	TOTAL NON PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg	mg	mg	mg	mg	vol per cent	
8	16 cc 3 per cent sodium taurocholate	0	12 1	24 8	520	81	1 6	34 7	Dog died in 30 hours Hemorrhagic pancreatitis, fat necrosis
		1	23 3	56 4	450	20	2 4	43 3	
9	22 cc 2 per cent sodium taurocholate	0	11 2	28 0	510	80	1 6	34 7	Dog died Hemorrhagic pancreatic necrosis
		1	14 0	24 8	500	52	1 2	39 5	

TABLE IV

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS  
PRODUCED BY INJECTION OF ETHER INTO DUCTUS SANTORINI

DOG NO	QUANTITY INJECTED	DAY AFTER OPER- ATION	BLOOD						REMARKS
			AMOUNT PER 100 C C					CO <sub>2</sub> COM- BINING POWER	
			UREA NITRO- GEN	TOTAL NON PROTEIN NITROGEN	CHLOR IDES	SUGAR	CREATI- NINE		
10	4 c c ether	0	9.3	29.7	530	71	13	38.5	Death 10th day Local abscess
		1	14.0	45.0	460	89	12	39.5	
		2	16.3	43.6	480	48	14	40.4	
		6	19.6	37.5	550	80	15	40.4	
		7	40.1	77.3	500	77	20	37.6	
		8	91.0	155.0	180	79	21	38.5	
		9	126.0	158.0	500	69	20	40.4	
		10	159.7	198.0	520	76	26	40.4	

TABLE V

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG IN EXPERIMENTAL ACUTE PANCREATITIS  
PRODUCED BY INJECTION OF GALL BLADDER BILE INTO DUCTUS SANTORINI

DOG NO	QUANTITY INJECTED	DAY AFTER OPER- ATION	BLOOD						REMARKS
			AMOUNT PER 100 C C					CO COM- BINING POWER	
			UREA NITRO- GEN	TOTAL NON PROTEIN NITROGEN	CHLOR IDES	SUGAR	CREATI- NINE		
			mg	mg	mg	mg	mg	vol per cent	
11	10 c c bile in- jected at 80 mm mercury pressure	0	9.3	24.6	510	80	12	38.5	Dog killed 30 days postoper- ative Pancreas beginning to atrophy
		1	16.3	36.6	510	88	14	39.5	
		2	12.1	27.0	520	86	14	40.4	
		6	18.6	33.0	520	84	14	39.5	
		10	23.3	41.6	490	73	14	37.6	
		15	23.3	41.0	510	83	12	42.4	
		24	32.6	56.0	480	96	14	39.5	
		27	12.1	33.0	500	83	14	41.4	
		34	9.3	28.5	520	62	14	39.5	
		38	14.0	30.0	530	79	12	40.4	
12	8 c c bile in- jected with B coli	0	9.8	21.8	520	68	14	39.5	Dog alive 24 days
		1	24.7	52.8	510	50	20	44.3	
		2	20.5	46.8	400	62	20	42.4	
		3	14.0	34.5	410	70	18	45.3	
		6	10.2	22.4	410	62	14	46.2	
		10	9.3	20.8	490	89	12	40.4	
		24	9.3	23.0	500	78	16	42.4	

The animals receiving gall bladder bile lived longer than those receiving sodium taurocholate. Not all of the animals showed definite blood chemical changes. The dogs receiving sodium taurocholate showed profound hemorrhagic pancreatitis followed by death within twenty-four to seventy-two hours. Neither the amount of pathology in the pancreas nor the blood chemical changes had any definite relationship to the pressure under which the materials were injected. The urea nitrogen and nonprotein nitrogen were increased in 12 of the 21 dogs receiving gall bladder bile, 3 of the 4 receiving ether, 5 of the 8 receiving infected

TABLE VI

CHEMICAL FINDINGS IN THE BLOOD OF THE DOG FOLLOWING INJECTION OF GALL BLADDER BILE INTO DUCTUS SANTORINI CHANGES WITHIN NORMAL LIMITS

DOG NO	QUANTITY INJECTED	DAY AFTER OPERATION	BLOOD						REMARKS
			AMOUNT PER 100 CC					CO. COMBINING POWER	
			UREA NITROGEN	TOTAL NON PROTEIN NITROGEN	CHLORIDES	SUGAR	CREATININE		
			mg	mg	mg	mg	mg	vol per cent	
13	4 cc bile	0	13.0	28.2	510	71	16	40.4	Dog killed No gross pancreatic changes
		1	16.3	31.6	450	69	16	38.4	
		2	14.0	32.6	480	71	14	40.4	
		6	13.5	29.1	530	74	16	40.4	
		10	10.2	29.4	520	61	13	40.4	
		18	10.2	27.7	550	79	12	36.2	
		33	15.8	32.6	510	77	12	36.6	
14	3 cc bile	0	9.3	28.5	510	83	14	38.6	Dog killed Pancreas appeared normal
		1	11.6	28.5	500	68	15	40.0	
		2	11.6	30.0	520	72	16	38.6	
		4	9.3	28.5	520	66	16	46.0	
		8	8.8	22.7	550	77	12	37.6	
		12	14.0	29.7	550	108	14	35.7	
		32	9.8	26.3	520	80	13	38.5	
15	6 cc bile	0	14.9	28.9	550	85	14	40.4	Dog died Pancreatic necrosis
		1	14.0	27.0	540	79	12	38.5	
		2	12.1	28.2	530	63	12	40.4	
		3	14.9	28.2	540	76	13	38.5	
		4	18.2	34.5	540	71	14	37.6	

bile, and 2 of the 3 receiving sodium taurocholate. The creatinine was increased in 14 animals. Seventeen animals receiving gall bladder bile showed no change in the chlorides, whereas, 5 of the 8 receiving infected bile showed a definite decrease. Two of the 4 receiving ether and 2 of the 3 receiving sodium taurocholate showed a fall in the chlorides. Nine animals of the entire series showed a rise in the carbon dioxide combining power and the remaining 27 showed no constant change. The changes in the blood sugar were not constant. An increase in the sugar content was noted in 10 animals, a decrease in 14 animals and no change in 12.

## SUMMARY

Sterile and infected gall bladder bile, sodium taurocholate and ether, when injected into the duct of Santorini, produced a definite pancreatitis or pancreatic necrosis of varying degree in all cases

The urea nitrogen and nonprotein nitrogen of the blood showed an increase in approximately two-thirds of the animals

The chlorides showed practically no change except a slight decrease in 13 instances

The carbon dioxide combining power showed a slight increase in 9 animals

Following operation there frequently occurred a decrease in blood sugar followed by hyperglycemia

The creatinine usually showed either a slight change or a terminal rise, which was not always proportional to the rise in urea and nonprotein nitrogen

The changes in the nitrogen elements closely simulate those found in experimental peritonitis, acute intestinal obstruction and dehydration. No explanation is offered for the blood sugar changes

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## ACUTE SPONTANEOUS GLOMERULITIS IN THE RABBIT\*†

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VARIOUS forms of spontaneous lesions of the rabbit's kidney have been described by many observers who have used this animal for experimental purposes. The one most frequently found is that of focal nephritis, characterized by destruction of tubules, and by an increased amount of connective tissue in which numerous glomeruli may usually be seen in a fair state of preservation. This lesion in its chronic form usually produces marked pitting of the surface of the kidney. Another lesion is one described by LeCount, in which there is a wedge shaped mass of dilated tubules, with its base at the surface of the kidney. A third type, described by Mallory and Parker, is acute glomerulonephritis, this is characterized by proliferation of the lining endothelium of the capillaries leading to occlusion of them. Several mitotic figures are found in a single glomerulus. The entire tuft is usually enlarged. The lesion is diffuse throughout the kidney. In the epithelium there is formation of hyaline droplets, necrosis, and calcification.

The lesion that I wish to place on record as occurring spontaneously in the rabbit is very much more acute than those mentioned, and occurs only in a small number of glomeruli in any part of the kidney. Not more than four affected glomeruli have been observed in any single section of the kidney.

In a study of the rabbit's kidney covering many years, I have observed these changes in only two animals. Protocols of these two rabbits are recorded herewith.

RABBIT 1—January 27, 1931, a rabbit weighing 1.4 kg. was catheterized. The urine contained many hyaline casts, a few pus cells and on culture innumerable gram-negative bacilli. Culture of a catheterized specimen of urine obtained the following day disclosed the same conditions. Four days later the animal died, apparently as the result of diarrhea, not as the result of any experimental procedure. At necropsy the urine from the bladder contained many pus cells and many gram-negative bacilli. Cultures were not made from the kidneys. The ureters and pelvis appeared normal except for a few leucocytes in the lining of the pelvis. The kidneys were brownish red, and appeared smooth after removal of the capsule. On gross section there was no evidence of chronic focal nephritis or other change.

Microscopic examination disclosed a number of foci of acute inflammation in the upper portion of the papilla and in the lower portions of the cortex. These portions were not related to the glomerular lesions found scattered through the cortex. Throughout both kidneys the glomerular tufts were filled with large numbers of polymorphonuclear leucocytes. They were uniformly distributed throughout the glomerulus or were present in groups in a few loops. Occasional leucocytes are seen in sections of glomeruli of normal kidneys but not in the number seen in all of the glomeruli of this animal.

The glomerular lesions are described in what appears to be their various stages

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of development. The most acute lesion is seen in Fig 1. About 20 polymorphonuclear leucocytes can be counted in one section of the glomerulus, in addition to a considerable amount of nuclear debris of probable leucocytic origin. The structure of the glomerulus has been almost entirely destroyed, only a small rim of tissue in one quarter suggests tuft structure. Most of the glomerular tuft consists of hyaline, pink staining material in which are seen many leucocytes, and large, clear nuclei of the cells lining the tuft. Cells with similar nuclei are seen in large num-

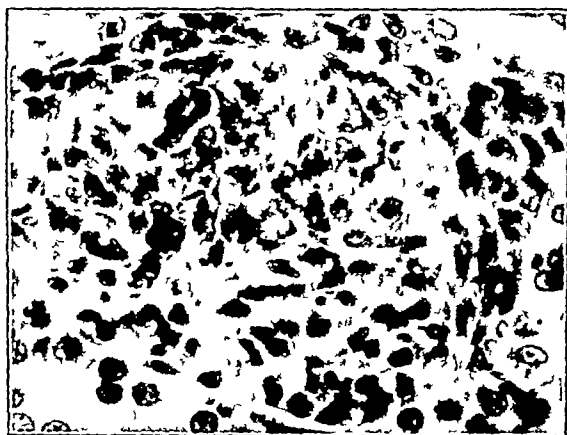


Fig 1—Marked infiltration with beginning necrosis

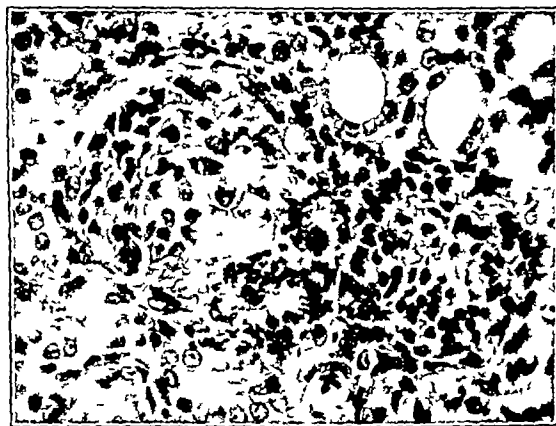


Fig 2—Acute polymorphonuclear infiltration with necrosis

bers in the wall of the thickened capsule on one side. Nuclear debris is most marked in the central portions of the glomerulus. There is no periglomerular infiltration. Fig 2 represents a second glomerulus, with a large mass of polymorphonuclear cells in one area, and in the opposite end of the glomerulus a few normal capillaries filled with erythrocytes. Two-thirds of the glomerulus has been entirely destroyed. The hyaline material does not contain leucocytes but does contain many large, clear nuclei which increase in number in the capsule. In a closely adjacent glomerulus, there are ten polymorphonuclear cells in the loops that impinge on the glomerular wall opposite the affected glomerulus.

In a little later stage (Fig 3) the leucocytes have diminished so that only five are seen in a section through an extremely large glomerulus. The destruction of the glomerulus is not as complete as in the first glomerulus described. About one-fourth of the glomerulus appears normal and the loop is filled with erythrocytes, in the other three-fourths the structure has been entirely lost and is made up of hyaline material, nuclear debris, and large, clear nuclei. The capsule is definitely thickened on the surfaces adjacent to the necrosis. Periglomerular in-

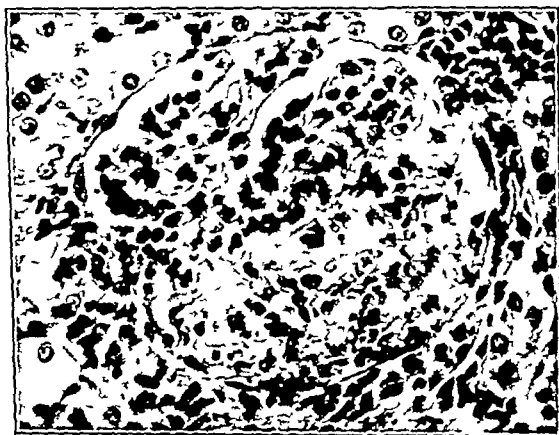


Fig 3—Subacute hyaline necrosis

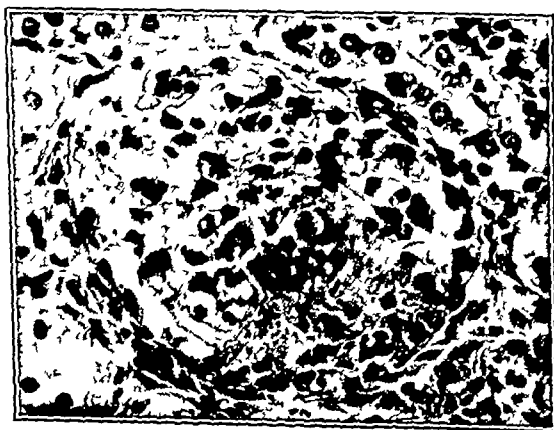


Fig 4—Destruction of one-fourth of a glomerulus

filtration with lymphocytes is present, and is most marked toward a small vein close by.

In still later stages the nuclear debris has almost entirely disappeared, and the glomerulus is made up of a mass of clear nuclei lying in a field of hyaline material completely filling the glomerular capsule. No periglomerular infiltration with lymphocytes was present about the glomerulus. Many sections were carefully searched, but older stages were not found in the kidneys of this animal.

RABBIT 2—December 2, 1931, a catheterized specimen of urine from a rabbit weighing 1.90 kg. did not contain albumin or casts, and on culture proved

sterile December 4, 1931, at 3 P M, the animal received 50 c c of 20 per cent sucrose solution containing some dead colon bacilli. The animal was found dead the following morning. The gross examination of all of the organs was negative. I have injected this mixture into many animals, without finding any renal changes similar to those to be described here, and since the interval between injection and death of the animal was too short to have given opportunity for the production of such changes it seems certain that they were spontaneous in origin.

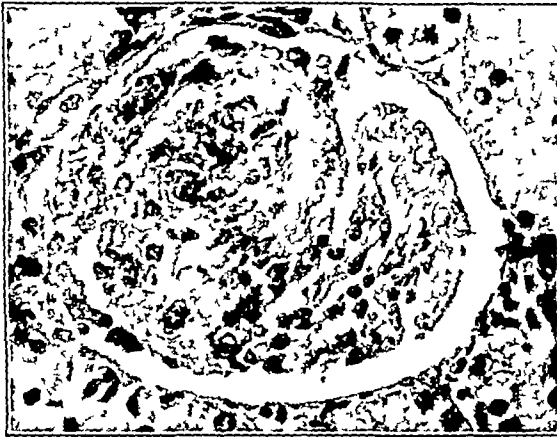


Fig 5—Necrosis no leucocytic infiltration

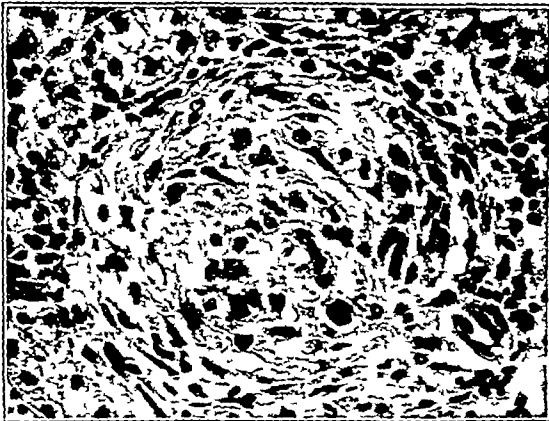


Fig 6—Chronic stage

Sections of the kidney disclosed the typical granular degeneration of the convoluted tubules that is constantly seen after intravenous injection of 20 per cent sucrose. There were a few areas of chronic infiltration in the papilla and cortex. The changes in the glomeruli were definitely less acute than in the first rabbit. They ranged from acute changes in which necrosis of a portion of the glomeruli was seen, to early fibrosis of the glomeruli. In the acute stage, from one-fourth to half of the glomerulus was destroyed, and the normal glomerular structures were replaced by a mass of pink-staining, granular material in which



were numerous small and large granules of nuclear debris. The number of nuclei in the glomerular capsule was definitely increased, and in one area it was made up of four layers of cells. As could be seen in the series of eight sections through this one glomerulus, the lesion was definitely limited to one of its portions. In the first section about half of the glomerulus was replaced by necrosis. In the second and third sections (Fig. 4) the necrosis involved the upper third of the glomerulus, with nuclear fragmentation most marked in the center of the glomerulus. In the fifth and sixth sections the necrosis was limited to about a fourth of the area. In the seventh section there was only a small area of necrosis, and in the eighth the glomerular loops appeared practically normal, but the capsule was thick and infiltrated with a number of lymphocytes. In Fig. 5 another example of acute glomerulitis is shown with destruction of about two-thirds of a glomerulus.

The more chronic stage of the lesions may be seen in Fig. 6. The necrotic material had been almost entirely removed, and in its place there was a concentrically arranged mass of epithelioid cells in which nothing of the former glomerular structure remains. There was some periglomerular infiltration with lymphocytes. This was the oldest lesion found in the kidneys of this rabbit. It is not difficult to conceive of this mass of tissue that has replaced the glomerular tufts gradually shrinking, and leaving the small, completely sclerotic glomeruli that are so often encountered in kidneys of the rabbit which otherwise appear normal.

#### COMMENT

In many kidneys which appear entirely normal, small, sclerotic glomeruli are found in a parenchyma that shows no evidence of other changes. The number of such glomeruli in any normal-appearing kidney in the rabbit is extremely small. Examples of the terminal stage must be present in larger numbers, and for a longer time, than the acute stages of the process that leads to this sclerosis. It is therefore not surprising that the acute stages of the development of this lesion have not been described heretofore. The change in the glomeruli is so striking that it is not likely to be overlooked, but since it occurs in only one or two glomeruli in the 800 to 1,000 glomeruli of a transverse section of the kidney, it is not likely to be found unless a careful and complete examination of a section is made. It seems probable that the acute and chronic changes observed in the glomeruli of these rabbits might result in the sclerotic glomeruli to be seen in many apparently normal kidneys of the rabbits.

In conclusion I want to emphasize again that these lesions were spontaneous in origin and that in the interpretation of experimentally produced acute glomerular lesions this source of error is to be considered.

## DIFFUSE ENDOTHELIOMA OF LYMPH NODES\*†

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ACCORDING to Flynn, the term endothelioma was advanced by Golgi in 1869. Chambard, in 1880, wrote a description of primary cancer of the lymph nodes arising from endothelial cells and to him is generally given credit for the discovery of this tumor. He noticed that this neoplastic process may be localized in a node or chain of nodes, or that it might assume a generalized form.

Hoffman and Schottlin, according to Benzinger, confirmed Chambard's finding by a case of their own. Recklinghausen in 1897 recognized primary endothelioma of the lymph node as a distinct tumor.

The next event in the history of this neoplasm was the publication in 1913 of Ewing's monograph on primary endothelioma of lymph nodes. In this paper Ewing reported a series of cases and claimed a definite clinical importance for the disease which had until that time been considered a medical curiosity. He noted that primary endotheliomas of the lymph nodes were commonly confused with such conditions as secondary carcinoma, lymphosarcoma and Hodgkin's disease, and pointed out wherein they differed histologically, anatomically, and clinically.

Oliver, also in 1913, published a paper on the relation of Hodgkin's disease to lymphosarcoma and endothelioma of the lymph nodes. He fully concurred with the opinions expressed by Ewing and included histologic descriptions, without clinical histories, of eight cases. Later authors on the subject of primary endothelioma of the lymph nodes have not added to Ewing's description and have made no new contributions to our knowledge of the subject. At the present time, however, pathologists do not as a rule attach the importance which Ewing did in his monograph to chronic granulomatous processes, notably tuberculosis, as an etiologic factor in the development of this tumor. Since 1913, primary endothelioma of the lymph nodes has been generally considered to be not uncommon. However, the relevant literature of the last two decades is surprisingly scanty considering the clinical importance of this neoplasm. Furthermore, the number of cases reported is remarkably few.

The incidence of the condition varies greatly at different medical centers. In this regard, Piney states that the great divergence of opinion regarding the nature of endothelioma accounts for the fact that some pathologists assert it is quite common, while others make the diagnosis of this tumor seldom and with hesitation. Dijkstra says that there are few tumors concerning which there is such a wide diversity of opinion. He notes that a tumor may be diagnosed as endothelioma by one author, another will diagnose a similar one as carcinoma, while a third will place the same type of neoplasm among the sarcomas or fibromas. He says that this difference in opinion is due in part to the unsolved problem of the structure and origin of endothelial cells.

\*From the Clinical Laboratory, Laboratory Center and Cancer Center, Edward Hines Jr. Hospital.

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TABLE I

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
Guaccio (quoted by Bittinger, 1926)	21	Female	Tumor size of hazelnut in right axillary region. Other left side of neck and left axilla	1 yr		Synechio endothehoma			
Battigelli, F., 1926	60	Male	Frontal headache tumor, painless and movable in left suprahyoid region. Incision in size. Nodules in pharynx covered with normal mucosa. Wassermann negative	Several years		Plasmocellular endothehoma	Biopsy	Two months or so later patient extremely cachectic. Left cervical, axillary, and epitrochlears of both sides enlarged. Nodules in pharynx so enlarged that patient nourished with milk. Spleen palpable. Lymphatic nodes not enlarged. No later report	At age of thirty left testicle enlarged and painful after a few months. Patient thought diagnosed tuberculous but had no further disturbance of genitourinary organs and a negative history until onset of present illness
Guthrie, A., 1920	33	Female	Numerous subcutaneous nodules infiltrating the external portion of left mammary and left pectoral areas. Mammogram glands negative. One nodule below right mammary gland			1st—subacute diffuse dermatitis (specific? leprosy?) 2nd—"Subcutaneous inflammatory lymphogranuloma"	Biopsy. K I Neo salvarsan X ray Radium	In spite of all treatment nodules continue to form (anterior abdominal wall). General condition became bad and patient confined to bed. No further details	
Flynn, C., 1927	26	Male	Painless enlarging lump at angle of lower jaw	7 mo	Adenitis due to infected teeth and tonsils	Primary endothehoma. Cervical nodes	X-ray therapy	Not affected by x-ray treatments. Death 2 yr and 6 mo after first nodule appeared. No more topography	In addition to tumor at angle of jaw, postcervical and suprascapular nodes became enlarged. X-ray showed enlargement of hilus and bronchial nodes. Metastasis to left breast

TABLE I (CONTINUED)

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
McCartney, J S 1928	15	Male	<i>First</i> <i>Admittance</i> Headache, weakness, dysp- pnea in legs, dysp- nea Bilateral cer- vical adenitis (most marked, right side)  <i>Second</i> <i>Admittance</i> Well marked cervical swelling (left side) Marked weight loss	4 wk	<i>First</i> <i>Admittance</i> Syphilis (posi- tive Wassermann) Infected tonsils and adenoids	<i>Second</i> <i>Admittance</i> Endothelioma	<i>First</i> <i>Admittance</i> Antisyphilitic therapy Removal of tonsils and adenoids  <i>Second</i> <i>Admittance</i> Biopsy of cervical nodes	Death a few months later Partial autopsy	Partial autopsy revealed large retroperitoneal nodes, and enlarged tri- cheobronchial nodes, all showing characteristic microscopic picture of primary endothelioma of lymph nodes
Zafargana, A 1928	73	Female	Painless tumor size of orange in left axilla	9		Primary endothelioma of axillary glands	Enucleation of tumor		Case showed a positive Wassermann Antinuclear treatment did not affect size of tumor
Lino, G 1927	21	Female	Small tumor (size of pea grew to size of hen's egg) in right latero cervical region General health excel- lent	6 mo	Neoplasia of lymphatic glands Endo- thelioma favored due to slow course	Primary endothelioma of lymph nodes	Operative removal of tumor		

TABLE I (CONTINUED)

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURATION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
Author's Cases Case 1 H W 1931	32	Male	<i>First Admittance</i> Small mass right neck (enlarged lymph nodes)	14 mo		<i>First Admittance</i> Lymphadenoma	Biopsy doses of deep x ray	No diminution in size of glands following x ray treatment Patient home in fair condition	At time of patient's second admittance he has well marked symptoms of thyrotoxicosis This latter condition will be treated before surgical treatment of tumor is attempted
			<i>Second Admittance</i> Shortness of breath, nervousness Bilateral enlargement of cervical glands Thyroid on right side enlarged	15 mo later		<i>Second Admittance</i> Slide reviewed with establishment of diagnosis of endo thelioma			
Case 2 W B 1931	36	Male	Large mass size of fist in left neck with chain of enlarged glands in neighborhood Small soft mass size of pea on left nasopharynx surrounding auditory tube	1 yr	Hodgkin's disease	Biopsy specimen taken from both the gland and mass in pharynx Diagnosis of endo thelioma made	X ray and radon implants followed by surgical removal	No response to x ray or radon implants	Patient's general condition excellent
						<i>First Admittance</i> Metastatic carcinoma			
Case 3 C S 1931	57	Male	Tumor left side of neck	of 5 yr	Sarcoma	<i>Second Admittance</i> Restudy of slide establishes endo thelioma	Deep x ray	No response to x ray therapy, general condition good	In last few years he has lost a moderate amount of weight General condition good No evidence of metastasis

TABLE I (CONTINUED)

AUTHOR AND DATE OF REPORT	AGE	SEX	SYMPTOMS	DURA TION	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	TREATMENT	CLINICAL COURSE	REMARKS
Case 4 J G 1931	62	Male	Complaints of a "cold" Shortness of breath, pain in chest, swelling in left supra- clavicular fossae	?	Malignant new growth of neck in the midline trunk	Biopsy of axillary gland reported as chronic adenitis Autopsy specimen diagnosed as en- dothelioma	Diagnosis	No response to x-ray therapy Symptomatic pro- gressive Death 7 mo after admission	Autopsy showed a tumor of anterior cervical nodes and those of the supraclavicular fossae on the left side The mass weighed 500 grams A portion of the tumor ex- tended behind the clav- icle, expanding in the mediastinum to surround the aortic arch The left lung was compressed and showed a nodule 1.5 cm in diameter with the same appearance as the tumor
Case 5 J H 1931	53	Male	Enlargement of neck on left side	3 1/2 yr	Hodgkin's dis- ease	First Admittance 1928 Hodgkin's Dis- ease Second Admittance 1931 Endothelioma	X-ray in 1928 with no response Sur- gical removal of mass in 1931, since a small subclavicular nodule which was inaccessible	No response to x-ray treatment Since surgical operation, patient is in excellent condition and has gained 8 pounds at the time of this report, 8 1/2 mo postoperative	

As noted by Flynn, the condition in its incipency is a process usually localized in glands readily accessible to surgery. At this stage, surgical intervention offers the best prospect of complete cure.

We present the following 5 cases which we have seen in a period of less than a year at the Edward Hines, Jr., Hospital. It will be noted that the majority of these patients had been under medical treatment for varying lengths of time before the true diagnosis was reached. Table I outlines from a clinical point of

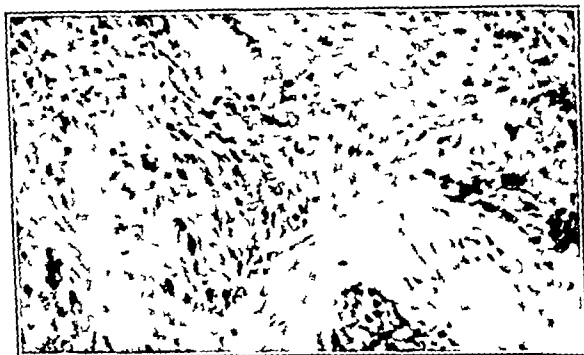


Fig 1—Sixteen mm obj cc 15× camera 50 cm ×300 Reduced Case 1 H W Diffuse type. Oval and polyhedral type cells growing in broad sheets completely replacing lymph node structures. Note the diffuse scarring.

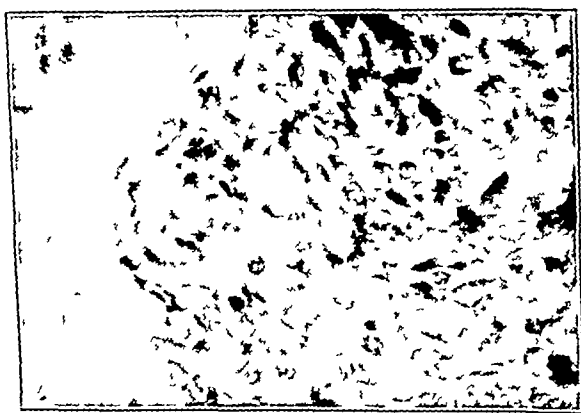


Fig 2—Eight mm obj cc 15× camera 5 cm ×600 Reduced Case 1 H W Detail showing indefinite cell outline.

view the cases of primary endothelioma of the lymph nodes which we have been able to collect from the literature since the publication of Ewing's monograph.

*Histopathology*—According to Ewing, endothelioma of lymph nodes may be divided into the three following groups:

- 1 Diffuse
- 2 Plexiform or perivascular
- 3 Alveolar

1 Those tumors which show round or polyhedral cells appearing in broad coherent sheets or syncytial masses comprise the diffuse group.

2 The plexiform type shows cells surrounding large irregular centers of cell

detritus or leucocytes. Occasionally cavities appear empty and are lined with flat cells resembling endothelium. Ewing interprets these cavities as derivatives of the original sinuses of the node but is not convinced that this is their sole origin. When the sinuses are highly developed, the tumor cells cling to the arterioles of the septa producing a characteristic perivascular arrangement which recalls the structure of a perithelioma. In the plexiform and perivascular tumors the cells usually assume a spindle form but readily change to polygonal or pavement types.

3 The alveolar type presents many difficulties of interpretation. It is seldom possible to trace the tumor cells to any structure of the original lymph node, and

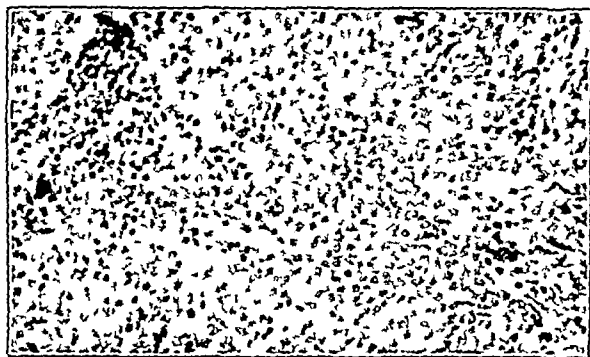


Fig 3—Sixteen mm obj c.c. 15× camera 50 cm × 300. Reduced. Case 4, J. G. Peplac. Replacement of lymphoid structure by diffuse growth of tumor cells. Case previously diagnosed as chronic adenitis.

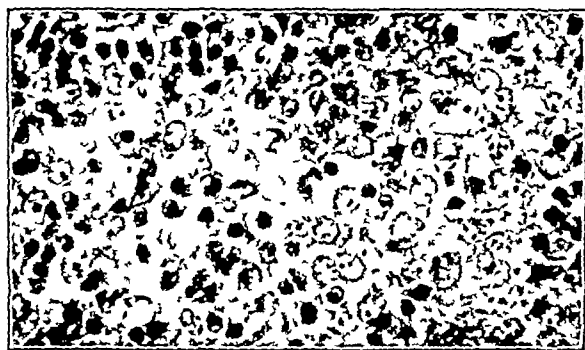


Fig 4—Eight mm obj 15× camera 50 cm × 60. Reduced. Detail showing vesicular character of cells arranged in broad sheets.

the cell groups often resemble those of secondary carcinoma. Thus the diagnosis of this type of endothelioma requires full clinical and anatomical data and must often remain uncertain. It is this type of tumor which has hitherto appeared in the literature and received considerable recognition as true endothelioma of lymph nodes. One of Ewing's cases demonstrates furthermore that endothelial proliferation associated with granuloma may early assume a form closely resembling alveolar endothelioma.

*Clinical Diagnosis*—Primary endothelioma of the lymph nodes may suggest from the clinical appearance acute infectious adenitis, tuberculosis, syphilis, lymphosarcoma, Hodgkin's disease, and, above all, metastatic carcinoma. Acute



infectious adenitis will usually show infected areas draining into the affected nodes. Pain and fluctuation eventually are characteristics of the condition. In this connection we may remark that tonsils and adenoids have been regarded as the source of adenopathy in several recorded cases of endothelioma of the cervical nodes, notably those of McCartney and Flynn.

Tuberculosis of glands usually occurs in patients whose history reveals an antecedent tuberculous infection and is characterized by a tendency to caseation and sinus formation. The characteristic temperature elevations and other sys-

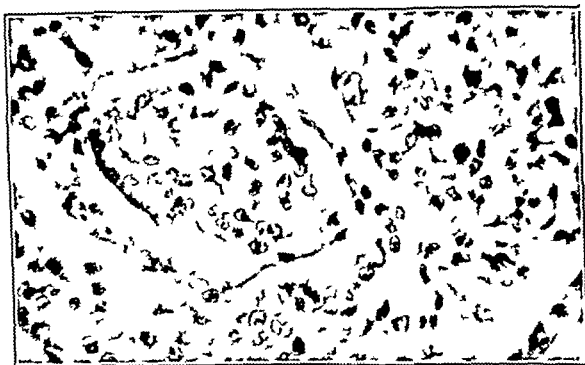


Fig. 5—Sixteen mm obj c.c. 15×camera 50 cm×300. Reduced Case 2 W. B. Note the alveolar like arrangement of cells.



Fig. 6—Four mm obj c.c. 15×camera 50 cm×1200. Reduced Case 2 W. B. Detail of alveolus revealing a cloudy indefinite cell cytoplasm and the highly vesicular character of the nucleus.

temic symptoms of tuberculosis may be expected. Flynn writes, in this regard, that when tuberculosis is manifested in cervical lymph nodes, it appears first in the submaxillary and soon afterward in the cervical nodes in both sides of the neck, growing slowly, and that gradually the postcervical, supraclavicular, scapular, and bronchial nodes become affected. On the other hand, in primary endothelioma of lymph nodes, the primary tumor is usually localized in one chain, usually the anterior cervical, on one side.

Syphilis is usually suggested by the history and Wassermann reaction. The Wassermann, however, may lead one astray as noted in the cases reported by

McCartney and Zafagnini, in which a positive Wassermann reaction and an endothelial tumor of the lymph nodes were coexistent

Lymphosarcoma is characterized by its rapid course and early involvement of associated chains of nodes. This tumor responds to radiation therapy.

Primary endothelioma of lymph nodes is frequently diagnosed clinically as metastatic carcinoma. This is easily understood when we note, as shown by Crowe and Baylor, that in a large series of cases, neck tumor is the first sign of carcinoma, primary in the nasopharynx, mouth, or accessory sinuses. This fact is also stressed by Quick and Cutler. As stated by these latter authors, the failure

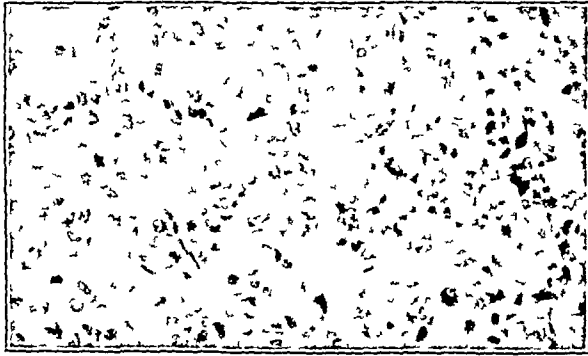


Fig 7—Sixteen mm obj c c 15×camera 50 cm×300 Reduced Case 3 C S Cells growing in columns with an arrangement resembling secondary carcinoma

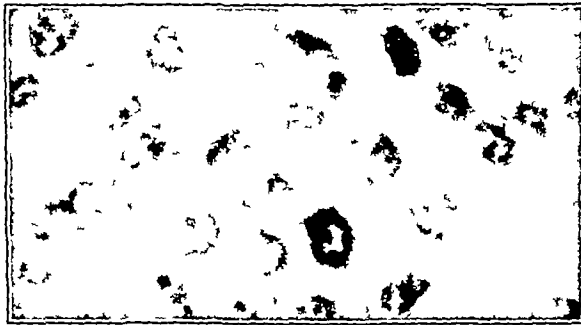


Fig 8—Four mm obj c c 15×camera 50 cm×1200 Reduced Case 3 C S Detail of the invading cells. Note the mitotic figure

after careful search to demonstrate a primary neoplasm, the rapid course of carcinoma, and the fact that it is radiosensitive should serve to distinguish it from endotheliomas.

Primary endothelioma of lymph nodes differs clinically from Hodgkin's disease in that in the latter a generalized adenopathy usually ensues shortly after the appearance of the cervical tumor. Hodgkin's disease is furthermore characterized by remissions while the course of endothelioma is slowly and steadily progressive. Hodgkin's disease readily responds to radiation.

We would conclude from a study of our own cases and from those reported in the literature that there are important distinctive features which are characteristic of primary endothelioma of lymph nodes. While, as noted by Quick and Cutler,

endothelioma of lymph nodes may occur as a systemic involvement of many nodes or as single or multiple tumors of the cervical, axillary, or other lymphatic chains. Typically the primary tumor involves and is localized in the anterior cervical group of nodes. The individual glands are usually described as remaining more or less discrete and are found to be rather elastic on palpation. The tumor mass is rarely painful.

The long duration of the clinical course with absence of systemic involvement, above all the lack of the cachexia so characteristic of malignant tumor is one of the



Fig 9—Eight mm obj c c 15  $\times$  camera 50 cm  $\times$  600 Reduced Case 5 J H The alveolar like arrangement of cells is noted



FIG 10—Four mm obj c c 15  $\times$  camera 50 cm  $\times$  1200 Reduced Case 5 J H Details of alveolus showing the characteristic endothelial type cell

salient features of this neoplasm. Thus, we note that in one of our cases (C S) at the end of five years the patient is in good general condition. In another case (W B) with a history of somewhat over a year's duration, the patient is in excellent general condition. In a third (H W) twenty-five months after the appearance of the tumor, the patient shows no evidence of a generalized involvement and is in fairly good physical condition in consideration of the fact that at the present time he has a thyrotoxicosis which will have to receive attention before the tumor is dealt with. In Flynn's case, which was not treated surgically, death resulted two years and six months after the first nodule was noted.

The third important point in the clinical behavior of this tumor is the fact

that it is not affected in any way by radium or x-ray therapy. This feature has been noted in all five of our cases and in those reported by Flynn and Guttierrez. Quick and Cutler likewise note that the lack of response of endotheliomas to radiation is an important feature in their diagnosis from a clinical standpoint.

We may note that in general the average age incidence of primary endothelioma of the lymph nodes appears to be somewhat earlier than that of carcinoma and is about the same as that for Hodgkin's disease and lymphosarcoma. There does not appear to be any particular sex distinction in the occurrence of this tumor. Obviously, no statement can be made from our observations regarding age or sex, as the patients who we observed are limited for the most part to veterans of the World War.

Despite the fact that the clinical features may be suggestive, the diagnosis is established by the pathologist after histologic examination.

*Prognosis*—Endothelioma of the lymph nodes unquestionably offers a better prognosis as regards length of life than does sarcoma, carcinoma, or the average case of Hodgkin's diseases.

In one case of our series the patient came to the hospital in what was evidently a terminal stage of the condition, and death resulted after several months. Three other patients are living and well at the end of periods of one year, five years, and three and one-half years respectively. The fifth patient, the known duration of whose tumor is over fifteen months, shows no ill effects from endothelioma, but has at the present time a thyrotoxicosis.

We realize that the postoperative period in our patients is too brief to permit us to venture a statement as to whether or not the tumor has yielded to surgery. We will note with particular interest the case of (H) in whom at operation it was revealed that a small substernal portion of the growth could not be removed. Eight months, however, have elapsed since this operation, and at this time the patient is in excellent health with no evidence of recurrence. Zaffagnini and Lino each report cases, subjected to surgical operation and well at the time of the report. These reports like ours were made shortly after the operation.

The prognosis in individuals suffering from this tumor is favored by the fact that metastasis is rare and occurs late in the course of the disease.

So far none of the four patients in our series who are living show evidences of metastases. Our fifth one, in whom there was extensive involvement and in whom the tumor weighed 500 gm., at autopsy showed but one small metastasis, 1.5 cm. in diameter, in the left lung. In Flynn's case a metastasis to the left breast was noted. The extent of metastases in this case, however, cannot be known due to the lack of an autopsy.

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## AN INSTANCE OF THYROID MEDICATION IN MERCURIAL NEPHROSIS\*

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THE following is a report of a case of acute mercury poisoning which was successfully treated by thyroxin. It should be, we think, of particular interest to those who have to deal with heavy metal poisonings.

### REPORT OF CASE

Mrs E C, aged twenty eight, weight approximately 110 pounds, was admitted to the Jefferson Hospital on April 10, 1930, at 12 30 P M with the history of having inserted one tablet of bichloride of mercury 7½ gr (0.49 gm) in the vagina as an antiseptic. In the past she had used, she said, bichloride douches without harmful effects, and did not know that the use of the solid tablet without water would prove injurious. The tablet was placed in the vagina at 6 P M on April 5, one hundred and fourteen hours previous to admission to the hospital. Symptoms of poisoning were noticed within fifteen minutes. She had pain and burning in the vagina, felt faint, and weak, began to vomit and had urinary frequency. Her husband noticed a "poisonous" odor to her breath. The next day she had many bloody, watery stools, and developed a sore mouth. There were no abdominal cramps. After an initial diuresis, urinary function ceased at noon April 6, four days before admission. The family physician saw the patient twenty four hours after the poison was taken. He instituted treatment at home, trying to force fluids, but the patient was unable to keep anything on her stomach. High sodium bicarbonate enemas were given. In the face of complete urinary suppression, persistent vomiting, and bloody diarrhea, the family physician thought it best to send her into the hospital.

The family history was unimportant.

The past history was essentially negative. There was no history of previous kidney trouble.

On admission the temperature was 100° F, the pulse 102, the respiration 20, the blood pressure 108 systolic and 60 diastolic. The patient was well nourished and appeared comfortable lying flat in bed. Sensorium was entirely clear. The physical examination was essentially neg-

\*From the medical service of the Jefferson Hospital.  
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ative except for an extremely foul breath, white sloughy patches over the mucous membranes of the mouth and pharynx, a generalized abdominal tenderness, an inflamed and swollen vulva, and a necrotic vaginal mucosa. There was no generalized edema. Reflexes were normal. Anuria had been present for four days. Urine, 25 cc obtained by catheter, showed one plus albumin, no sugar, casts, red or white blood cells. The hemoglobin was 90 per cent (Sahli), red cells 4,590,000, white cells 25,650 with increase of polymorphonuclear neutrophils to 83 per cent.

#### TREATMENT AND COURSE IN THE HOSPITAL

The patient was seen too late even by the family physician to prevent absorption. Seven and one-half grains (0.49 gm.) of mercuric chloride had been placed in the vagina five days before admission to the hospital but no douches had been given. Conditions, it would seem, were almost ideal for complete absorption of the whole tablet. Surgery, we felt, had little to offer. Renal decapsulation certainly has not fulfilled expectations, and its results appear very doubtful. The question confronting us was, what treatment were we to use?

After absorption has taken place one would like, of course, to neutralize the toxin. Many chemical detoxicants have been tried and there are many conflicting reports as to their value. Zeigler's comment that recovery apparently takes place independently of the administration or the failure to administer any of the so-called specific chemical detoxicants seems well taken. In 1924, Dennie and McBride<sup>1</sup> stimulated a great revival of interest in chemical detoxication by emphasizing the success of sodium thiosulphate in the treatment of heavy metal poisoning. Careful consideration of their work, however, has led to skepticism in regard to their conclusions. Numerous men have found it ineffective in acute mercury poisoning. Haskell, Henderson and Hamilton,<sup>2</sup> for example, in experimental dogs poisoned by bichloride of mercury, found that sodium thiosulphate failed to prevent or even delay the effects of minimum lethal doses. Skepticism regarding the efficacy of sodium thiosulphate in mercury poisoning is, therefore, rather wide spread. It should never be used to the exclusion of the other well known and tried therapeutic measures.

In this case we adopted a method of treatment which seemed to combine the more effective measures of the various methods previously used. On the day of admission the patient was given 1,000 cc of 10 per cent glucose in 2 per cent sodium bicarbonate solution intravenously in divided doses of 500 cc each. Alkaline douches and enemas were ordered as local and eliminative measures. On the following day 400 cc of blood was withdrawn and 1,250 cc of 10 per cent glucose in 2 per cent sodium chloride solution given in divided doses. Alkaline diuretic mixture and milk were alternated every two hours by mouth. A sweat bath was given and hot packs were applied to the kidney regions. Magnesium sulphate was given by mouth and as rectal instillations. Eliminative and supportive treatment was adhered to very rigidly throughout the course of her illness. The intravenous salt and sugar solutions used, as well as the daily output of urine, are shown in Chart 1. Varying percentages were used in an effort to promote kidney action. Insulin was used along with the glucose from time to time. The alkaline therapy, sweats, and magnesium sulphate were continued. But the patient lost ground in spite of all treatment. The nonprotein nitrogen and creatinine were steadily rising and the urine output was not satisfactory. She became irrational, quite de-

luous, restless, and had several hard convulsions. The outlook for the immediate future seemed very gloomy, in fact, every one felt that the case was hopeless.

On the basis of favorable reports from the use of thyroid extract in nephrosis and in view of the profound metabolic disturbance in mercurial intoxication, thyroid therapy, we felt, might offer some hope for her. On April 23, thyroid therapy was started. Thyroxin gr 1/40 (16 mg) was given subcutaneously in order to secure immediate effect. This dose was repeated on April 24 and 26. Desiccated thyroid gland was also given by mouth up to 6 gr a day for seven days. One cannot be certain, though, as to how much of this was retained and absorbed, as the patient was vomiting a great deal. Signs of increased thyroid activity became evident. The pulse rate was more accelerated and definite exophthalmos developed. All

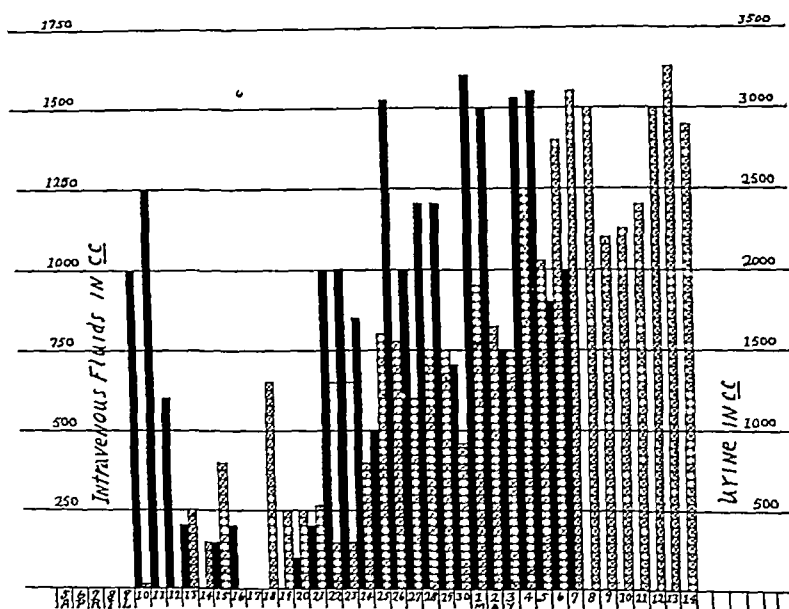


Chart 1—Intravenous fluids (black area) and urine chart (shaded area). Glucose 10 per cent in Ringer's solution was given except on April 13, 15, 16, 20 and 21 when equal parts of glucose 50 per cent and sodium chloride 30 per cent were used.

the other therapeutic measures that had been used previously were continued. On the second and third days after instituting thyroid therapy, the urinary output increased and continued until recovery became apparent and certain. Definite clinical improvement was evident within three days. Indeed, the whole picture changed from a seemingly hopeless one to an encouraging one, and the patient made a rapid recovery.

**Laboratory Data**—The specific gravity of the urine was low throughout, ranging from 1.001 to 1.011. The albumin was heavy at first and gradually decreased in amount, there being only a trace on discharge. It is interesting that no casts were ever found. There was a trace of blood microscopically in a few of the voided specimens and a fairly constant trace of pus. Epithelial cells were present in great numbers. There was sugar in the urine at times, probably due to the therapeutic measures. No acetone bodies were found and the reaction of the urine was

usually alkaline. There was no excretion of phenolsulphonephthalein on May 7, nor on May 12, but a trace in the two hours on May 19. Four months later there was 70 per cent excretion of the dye in a two hour period. Urine examination at this time was negative.

The blood chemistry was interesting. Daily determinations were made of non protein nitrogen, creatinine, and chlorides. The nonprotein nitrogen reached 288 mg per 100 c.c. of blood and the creatinine 10 mg per 100 c.c. of blood. The two curves ran parallel and each reached its maximum on the same day. These figures would, no doubt, have been much higher had not large quantities of intravenous fluids been given. The blood chlorides were low on admission, being only 265 mg per 100 c.c. of blood. The saline intravenously raised the level to about 400 mg and it kept around this point.

The blood picture changed markedly during the course of her illness. A severe secondary anemia developed. The hemoglobin went down to 31 per cent (Sahl), red cells 1,910,000 on May 21. The hemoglobin was 53 per cent with 2,900,000 red cells on May 9 at a time when the patient already showed marked clinical improvement. It is interesting that the anemia progressed so far after every other sign of improvement became evident. As stated above, there was a marked leucocytosis of 25,650 with an increase of the polymorphonuclear neutrophils to 83 per cent. Weiss<sup>3</sup> states that the degree of elevation of the white count corresponds closely with the severity of the symptoms of intoxication. This leucocytosis, he says too, may reach 20,000 and then decline, or remain elevated and carry an unfavorable prognosis. But in this particular case the leucocyte count was not followed closely. The blood picture improved rapidly on liver extract and iron. The basal metabolism was plus 2 on discharge, May 25, 1931.

#### DISCUSSION

The intravenous use of a hypertonic salt and glucose solution, consisting of equal parts of 50 per cent glucose (solution) and 30 per cent salt (solution), caused a moderate diuresis. But this did not persist. Fairly large quantities of a 10 per cent glucose solution failed to produce a diuresis in the beginning. However, intravenous fluids were continued chiefly in the form of 10 per cent glucose in Ringer's solution. The bicarbonate glucose solution and insulin lessened the acid intoxication which has been found to be constantly present in acute mercuric chloride poisoning. The beneficial effect of this is obvious, as delayed kidney injury is associated with the development of this acid condition.<sup>4</sup> A hypochloremia is constantly present in acute bichloride intoxication caused by the persistent vomiting and loss of fluids by bowel.<sup>5</sup> The saline and Ringer's solution raised and maintained the blood chlorides at a satisfactory level. The large quantities of intravenous fluids undoubtedly held the concentration of the nonprotein nitrogenous substances at a lower level. This may be explained by the factor of dilution, by the diuresis produced, and by the ability of carbohydrates to spare body proteins.

Mercury is excreted largely by the bowel and to a less extent by the kidneys, skin and other mucous surfaces. Magnesium sulphate by mouth and by rectum seems an important eliminative measure. The value of the sweat bath is hard to estimate. Lambert and Patterson<sup>6</sup> attached a great deal of importance to it.



Thyroid medication appeared to be very definitely a life saving measure in this case. In regard to its beneficial effect nothing definite can be said concerning its mode of action. However, the pronounced increased urinary output immediately following the administration of thyroxin suggests strongly that the thyroxin may well have been a factor in hastening the return of renal function. Further studies along this line, then, would seem desirable. During the course of treatment with thyroxin and thyroid extract the interesting observation was made of the experimental production of bilateral exophthalmos.

The phenolsulphonophthalein elimination in cases of acute bichloride poisoning presents a most interesting problem. It has been observed that the dye fails to be eliminated during the acute phase of the disease and during early convalescence. But within a relatively short period of time after recovery is assured, the kidneys eliminate normal amounts of the dye in the absence of preexisting nephritis. In the case under discussion there was no elimination of the dye on May 7, nor on May 12, and barely a trace in all specimens on May 19, five days before her discharge. Four months later there was normal phthalein elimination, 70 per cent in two hours. This case demonstrates clearly that this test is not a reliable index of kidney function during the recovery phase of acute bichloride poisoning.

In consideration of the facts observed in this case and from the histopathologic picture in the acute bichloride kidney, one may conjecture as to the most probable mechanism of phthalein excretion in the human kidney. It has been shown that the glomerular units are histologically intact and appear normal while the tubules show most extensive necrosis of the epithelium. Such cases furnish beautiful examples, it seems to us, of localized injury with respect to the kidney. It would seem, too, that much could be learned about the function of the various units by careful study of these cases. If the glomeruli were concerned particularly with the phthalein elimination, one would expect its appearance in the urine unless reabsorption were complete in the tubules, or unless no free phthalein existed in the blood, it being in a bound and nonfiltrable form. The first of these assumptions is unlikely and the latter has been demonstrated untrue.<sup>7</sup> Since there is no detectable dye in the urine, the data at hand points, in our opinion, to the tubules as the main seat of phthalein elimination in the human kidney, as Marshall<sup>8</sup> and his collaborators maintain.

Acknowledgment is made to Dr. Lawson for the privilege of reporting this case on whose service it was treated.

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# OUTLINE OF A METHOD FOR THE DETERMINATION OF THE STRENGTH OF THE SKIN CAPILLARIES AND THE INDIRECT ESTIMATION OF THE INDIVIDUAL VITAMIN C STANDARD\*†

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THE strength of the skin capillaries can be systematically ascertained by a test which is an extensive elaboration of the clinical capillary test used by C. Leede,<sup>1, 2</sup> Alfred F. Hess,<sup>3, 4</sup> R. Stephan,<sup>5, 7</sup> and others. The following modifications had to be made in the clinical capillary test to transform it into a test of the strength of the capillaries, satisfactory from the physical point of view.

The veins of the upper arm must be subjected to manometrically measured pressure, and in most cases *more than one degree of pressure* must be applied to make the determination. Each degree of pressure applied should be lower than the diastolic blood pressure in the brachial artery of the subject of examination. Otherwise the afflux of arterial blood is also partly obstructed. Finally, the skin area within which the observations are to be made should be definitely limited as to position, shape, and size.

In the author's method, pressures of 35 and 50 mm Hg were selected. The skin area to be examined is circular, with a diameter of 60 mm, and its center coincides with the center of the hollow of the elbow. In order to obtain sufficient sensitiveness in this test, it is necessary to maintain the pressure for fifteen minutes. In carrying out this test it proved advisable to fix limits between the various grades of strength in the following manner, according to a descending scale.

Grade I. No petechiae within the examined skin area at a pressure of 50 mm Hg in fifteen minutes.

Grade II. Petechiae appear at a pressure of 50 mm Hg, but their number does not exceed 6.

Grade III. More than 6 petechiae appear at 50 mm Hg, but none or at most one at 35.

Grade IV. At least 2 petechiae are present at a pressure of 35 mm Hg.

As we know, A. F. Hess<sup>3</sup> found that his "capillary resistance test" is positive in scurvy and he also states that it forms a clue in the diagnosis of latent scurvy, a conclusion which is confirmed in a paper of H. Ohnell.<sup>8</sup> But where Hess applied the test only in cases of scurvy, manifest or latent, the author has gone a step further and inquired into the possibility, by means of this test, of revealing much milder deficiencies in vitamin C, viz., such as lie between the smallest discernible deviation from a normal vitamin C level and the highest deviation possible without giving rise to actual symptoms of disease. The latter class together with cases of latent and manifest scurvy form a complex to which the author suggests giving the general name of *vitamin C undernourishment*.

The author determined the serviceability of the capillary strength test as a criterion of even low degrees of vitamin C undernourishment by the following process:

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†As to the results already arrived at by this method, more detailed information is available

in the author's paper: "A Method of Establishing the Vitamin C Standard and Requirements of Physically Healthy Individuals by Testing the Strength of Their Cutaneous Capillaries," *Skandinavisk Arch f. Fysiol.* 61: 225-270, 1931.

Two physically healthy adult persons were placed on a scorbutic diet which was supplemented by precisely measured daily rations of juice freshly squeezed from yellow Mediterranean oranges, and these daily rations were altered by progressive steps in the course of the investigation. It was ascertained (in January and February, 1930) that the original normal strength of the skin capillaries of the subjects of experiment fell to an abnormally low level when the ration of anti-scorbutic juice was kept low for weeks, but that this change in the capillary strength was reversible, the strength returning gradually to normal when a sufficient amount of juice was given for a sufficiently long time (several weeks).

By the testing of the capillary strength both of healthy vegetarians and of school children who had been suffering from vitamin C undernourishment, but whose vitamin C standard was raised by the eating of oranges (after which new capillary strength tests were made), the grades of capillary strength corresponding respectively to a normal and a subnormal vitamin C standard were ascertained. By this investigation, the capillary strength test was actually elaborated into a *nutritional hygiene test by means of which, in a great many cases, individual vitamin C standards can at any time be indirectly ascertained*.

A determination of the strength of the skin capillaries of an individual may, of course, be made whether he is healthy or not. In general, however, such a determination does not offer a solid foundation for conclusions as to the vitamin C standard unless the person examined is entirely healthy with the sole exception of possible symptoms of lowering of his vitamin C standard. Nevertheless, in some not unimportant diseases ("Points to be noted," No 3, 4 and 7), conclusions as to the vitamin C standard seem to be justified, although the conditions just mentioned are not fulfilled.

## I DESCRIPTION OF APPARATUS

The principal instruments for ascertaining the capillary strength are the following (Fig 1) \*

1 A *rubber stamp* (a) and ink pad for imprinting on the skin of the hollow of the elbow a colored ring within which the capillary observations are to be made. The ring is stamped so that its center coincides with that of the elbow. For adults and children down to two years old, a stamp making a ring 60 mm in diameter is used, and for younger children, one making a ring 40 mm in diameter. If the small stamp is used, the corresponding number of petechiae in a circular area measuring 60 mm in diameter can be computed by multiplying by 2.25.

2 A large *Politzer rubber syringe* (b), with an air-tight connection with its nozzle.

3 A *screw compressor* (c), so constructed that when its compressing surfaces of flat metal are farthest apart, the fully distended rubber syringe will fit in between them without distortion, while turning of the screw as far as it will go will completely compress the syringe.

4 A *rubber tourniquet* (d), of the kind used in measuring the blood pressure, for producing an external pressure on the veins of the arm. For adults and children down to the age of about ten, the rubber part of the tourniquet should be 12 cm wide, for children between nine and five, 8 cm, and for still smaller children,

\*The complete apparatus for the test of capillary strength herein described may be obtained from Kirurgiska Instrument Fabriks-aktiebolaget Stockholm Sweden.

5 cm The canvas part of the tourniquet may not extend over the edge of the rubber part for more than a few millimeters

5 A *low mercury manometer (m)* with conspicuous marks on the scale at 35, 50, and 65 mm Hg

6 A Y-shaped metal tube and air-tight, flexible rubber tubes, by means of which the air chamber of the tourniquet, the syringe in the compressor and the inlet tube of the manometer may be joined in a single closed air system

7 A *magnifying lens (e)* of 5 D with which to inspect the marked off skin area for possible petechiae

8 A circular *plane glass (h)* set in a metal rim with a handle, for locally pressing the blood out of the skin By this, it is possible in cases of doubt to distinguish between merely hyperemic red spots and genuine petechiae Hyperemic spots will disappear on pressure, whereas petechiae will become more distinct

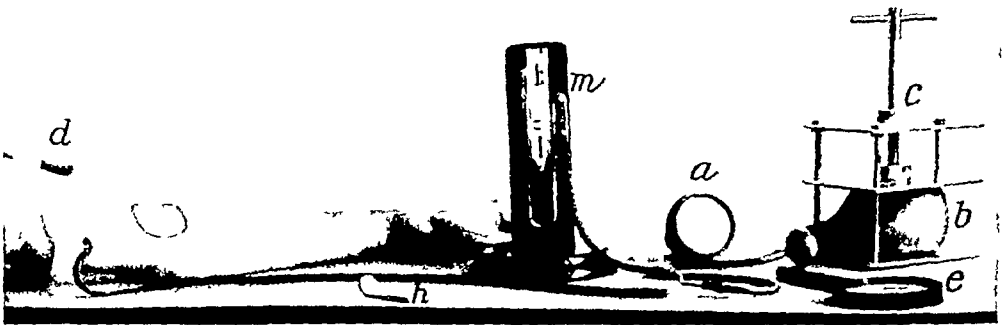


FIG 1

## II FURTHER REQUIREMENTS FOR MAKING THE TEST

1 In order to place the arm on a table in the plane of the heart, either an adjustable chain with back support, or a suitable number of wooden boards

2 It is convenient to employ a block of wood for raising the manometer above the level of the table, thus facilitating the reading of the mercury meniscus When the apparatus is set up for use, the tubes of the manometer should be strictly vertical

3 A watch with a second hand or preferably a stop-watch

4 Glass tubes with capillary points, black ink to indicate the petechiae and green ink for other spots with which the petechiae might otherwise be confused, and for any petechiae which in exceptional cases may be present before the application of pressure

## III HOW TO MAKE THE CAPILLARY STRENGTH TEST

During the test, the skin area to be inspected and consequently the surrounding air as well should have the proper temperature It has been found that an increase in the temperature of the skin (causing dilatation of the vessels) tends to increase the number of petechiae, while a lowered temperature (causing contraction of the vessels) tends to decrease the number of petechiae In cases of more pronounced deviation from the normal temperature of the skin, therefore, the results

obtained by this method will not be reliable. In view of this influence of temperature, the following precautions are necessary:

- 1 The temperature of the room should be between 16° C and 21° C
- 2 The subject must not have had a hot bath or a steam bath on the day of examination, nor have taken part in any sports, nor, within the three hours immediately preceding the examination, done any gymnastic exercises
- 3 In the winter, if the outdoor temperature is low, the subject must have been indoors in the required temperature sufficiently long not to feel in the least cold

The test should be carried out in broad daylight or in *exactly equivalent* artificial light, e g, that of a daylight lamp of sufficient strength. (The petechiae are even more distinct in green light than in daylight.)

The subject is placed in a comfortable chair with support for his back. (Examination in the recumbent position is also practicable, but would probably seldom be chosen.) One of the arms is bared to the armpit, care being taken that the rolled sleeves exert no pressure on the arm. Any garment doing so should be removed. The arm is then placed horizontally *in the plane of the heart*. This precaution is very important if a reliable result is to be obtained. If it is neglected, the absolute pressure in the capillaries tested may vary even though the manometer records the same level of pressure. If, for instance, the arm is placed 10 cm below the level of the heart, there will be besides the pressure registered on the manometer, an additional hydrostatic pressure of  $\frac{100 \times 1.058}{13.6}$ , equal to 7.8 mm Hg, and this pressure will also affect the capillaries during the test.

With the subject sitting down, the arm is most accurately placed when the surface to be examined is in the plane of the insertion of the third rib into the sternum. This can be done either by placing the arm directly on the table and adjusting the trunk by means of a swivel chair, or by supporting the arm at the desired level above the table on top of a suitable number of boards.

During the entire test, the subject must keep his arm as well as the rest of his body perfectly quiet.

A colored ring is imprinted with the rubber stamp on the arm with its center coinciding with the center of the hollow of the elbow. The area of the skin thus outlined must be carefully and thoroughly inspected through the lens before pressure is exerted. If one or more formations which may later be mistaken for fresh petechiae (such as small areas of hyperemia, capillary ectasiae, or remains of petechiae from previous tests) are visible, they should be carefully indicated, most suitably by green ink, before the test is made.

The rubber tourniquet is then wound tightly around the arm and fixed, care being taken that *the lower edge of the tourniquet is at least 2.5 cm above the nearest part of the colored ring*. Petechiae often appear in greater numbers just below the lower edge of the tourniquet rather than further away, and the distance between should therefore not be too small.

The compressor screw should be turned *as quickly as possible*, the pressure in the tourniquet and consequently the external pressure on the veins of the upper arm being thereby raised to the desired level (35, 50, or 65 mm Hg). At the moment the mercury meniscus in the manometer reaches the proper level, the time is

taken on the stop-watch started. The pressure is then kept constant for fifteen minutes if necessary by adjustment of the compressor.

When the pressure is released nothing should be done until the cyanosis of the arm has disappeared, this may be hastened by the raising of the arm for fifteen seconds. *The petechiae should be counted within half an hour after the release of pressure.* Within an hour some of the petechiae may already have disappeared. When the number of petechiae in the ring exceeds 3 it is advisable to indicate them by making a black ink spot close to each petechia after which the spots are counted. If the number is very great it is best to divide the circle into quadrants by two lines passing through the center at right angles to each other; the petechiae in only one of these quadrants need then be counted and multiplied by 4.

#### IV CONCLUSIONS FROM THE CAPILLARY STRENGTH TEST

In order to ascertain by the method in question to which grade of strength the capillaries of a person belong, we proceed in the following manner:

One of the arms is subjected to a pressure of 50 mm for fifteen minutes. If no petechiae appear the capillary strength is of Grade I. If a pressure of 50 mm gives up to 6 petechiae, it is of Grade II\*. If a pressure of 50 mm produces more than 6 petechiae, a new test should be made on the other arm with a pressure of 35 mm. If no petechiae, or not more than one appears, the capillary strength is of Grade III. If two or more petechiae appear, it is of Grade IV.

The relation between the strength of the skin capillaries of a healthy person determined in this manner and his vitamin C standard at the time of the test has been experimentally ascertained for the Nordic race only (cf G F Gothlin<sup>2</sup>). In 1930, the Hygienic Section of the League of Nations was asked to take the initiative in an investigation of this matter along the same lines as in the paper cited in the case of other races living in temperate climates for whom such an investigation might be valuable.

In my investigation the following was found to be true of healthy members of the Nordic race:

*If the technique outlined is adhered to and a pressure of 50 mm for fifteen minutes does not produce more than 1 petechiae in the encircled area, we may conclude that the vitamin C standard is normal.*

*If 50 mm Hg produces more than 8 petechiae or if 35 mm Hg produces more than one petechia, the vitamin C standard is definitely inferior.*

Since by this method it is not possible to draw definite conclusions as to the vitamin C standard from differences of less than 4 in the number of petechiae within the outlined area at a pressure of 50 mm, cases with from 5 to 8 petechiae are regarded as *transitional*, 5 or 6 petechiae rather suggesting a normal standard, and 7 or 8 a subnormal one, though owing to the limits of error involved by the method definite conclusions cannot be drawn.

#### V POINTS TO BE NOTED

1. In investigations on a large scale (as in schools, regiments, and the like) into individual vitamin C standards or average standards on certain diets, a good deal of time can be saved by connecting the tourniquets of three persons at a time

\*In the examination of members of the Nordic race it is very unusual for more than one petechia to appear at 35 mm Hg in a case in which there are only 6 or less at 50 mm Hg.

to one and the same manometer and compressor. For this purpose a tube with 6 branches is substituted for the Y tube. To the sixth branch is coupled an air blowing pump (like those used in blood pressure determination).

When the pressure has been raised by the aid of this pump almost to the required level, the pump is shut off from the air system by the application of a clamp on its rubber tube. By means of the compressor the pressure is exactly adjusted and maintained. In this way, three people are subjected simultaneously to the fifteen minute period of compression.

2. If a series of tests on one person is desired, it is necessary to allow at least a fortnight, but preferably a month to intervene between each test so that the ruptured vessels may have time to heal perfectly. By the testing of alternate arms, it is possible to have a month intervening between tests on the same arm and yet have new tests every fortnight. More frequent tests than this are hardly worth while.

In such series of tests, a careful inspection of the skin before the test, is of especially great importance. When only one arm is tested, the 35 mm. pressure should be applied *before* that of 50 mm. and it is desirable that at least an hour be allowed to elapse between these two tests.

3. It is remarkable that simple arteriosclerosis does not by itself reduce the strength of the skin capillaries, at least to judge from the observations hitherto made on a small scale (10 cases). This method apparently can therefore also be used to determine the vitamin C standard of persons suffering from simple arteriosclerosis, but otherwise healthy.

4. Since there may be cases of latent tuberculosis among those tested in a mass investigation, it is interesting to note that tests made on a small number (12 cases) of persons with manifest pulmonary tuberculosis do not support any assumption that tuberculosis alone reduces the capillary strength.

5. In various acute infectious diseases (such as measles and scarlet fever), a reduction in the strength of the skin capillaries is found<sup>1, 2, 3</sup> and may persist in many cases for a rather long time, but the cause of this reduction has not yet been investigated. In the case of persons who have within the two months immediately preceding the investigation suffered from an acute infectious disease, it is therefore, at the present stage of our knowledge, wisest not to estimate the vitamin C standard from the strength of their capillaries.

6. No such estimate can be made in albuminuric conditions either, since these appear generally to reduce the strength of the skin capillaries.

7. In cases of achylia and uncomplicated afebrile stomach ulcers the vitamin C standard may be estimated from the results of the capillary strength test.

When inferior capillary strength is found in persons suffering from achylia or chronic enteritis, it should be kept in mind that part of the vitamin C in the food may be lost, in the former cases through the unfavorable conditions for the preservation of that vitamin in the digestive canal (alkaline reaction), and in the latter because of the difficulties in resorption of the vitamin, which are presented by the catarrhal alteration of the mucous membrane.

#### SUMMARY

1. A test is described which makes it possible to determine the degree of strength of the skin capillaries in the hollow of the elbow.

2 The capillary strength, ascertained by this method, indirectly reveals the vitamin C standard of persons who are quite healthy or exhibit only such deviations from health as are in themselves due to a low vitamin C standard

3 It is suggested that with this same method it is probably also possible to estimate the individual vitamin C standard in cases of uncomplicated arteriosclerosis, afebrile tuberculosis, achylia, and uncomplicated afebrile stomach ulcers

4 This method may be used as a test of the individual vitamin C standard by physicians, hygienists, and dentists in their practice. It can also be used in the statistic examination of groups (e. g., in the army, in boarding schools, in orphanages, in old people's homes, in asylums, and in prisons) in order to ascertain whether the diets in use provide a sufficient supply of vitamin C

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## TWO CASES OF ANEMIA OF PREGNANCY WITH THE CLINICAL FINDINGS OF MYASTHENIA GRAVIS\*

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A LARGE number of cases of pernicious anemia of pregnancy have been reported in the literature and the symptoms, signs, and blood findings have been clearly stated by Schmidt,<sup>1</sup> Larrabee,<sup>2</sup> Allan,<sup>3</sup> Rowland,<sup>4</sup> McSwinnery,<sup>5</sup> Balfour,<sup>6</sup> and others. The signs and symptoms most frequently present are weakness, breathlessness, palpitation, pallor, and gastrointestinal disturbances such as nausea, vomiting, and diarrhea. There is usually a history of fever or its presence during the course of the disease. These signs and symptoms may be accompanied by a blood picture of a primary (Schmidt,<sup>1</sup> Balfour,<sup>6</sup> etc.), hemolytic (Minot,<sup>7</sup> Rowland,<sup>4</sup> etc.), secondary (Osler,<sup>8</sup> Evans,<sup>9</sup> Mills,<sup>10</sup> etc.), or aplastic (Larrabee<sup>2</sup>) type of anemia. The pallor is usually marked and icterus may or may not be present depending upon the type of anemia.

The percentage of cases in which the liver and spleen have been found enlarged has varied in the different series of cases reported. In Balfour's<sup>6</sup> series of 150 cases

\*From the Lilly Laboratories for Clinical Research, Indianapolis City Hospital, and the Department of Medicine, Indiana University.  
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the liver was enlarged in 8 per cent and the spleen in 18 per cent, while in 10 cases reported by Lariabee<sup>2</sup> the liver was enlarged in 16 per cent and the spleen in 70 per cent. The degree of asthenia has also varied. Wills and Mehta<sup>11</sup> in India remark



Fig 1—A photomicrograph of a section of the gastrocnemius muscle obtained at biopsy from Case 1. There is a marked variation in the size of the muscle fibers the larger ones appear granular while the small fibers have a hyaline or homogenous appearance. In both the cross striations are absent. In the intermediate sized fibers the cross striations are present in some areas. The blood vessels appear normal and there is no perivascular infiltration. Magnified 300 diameters.

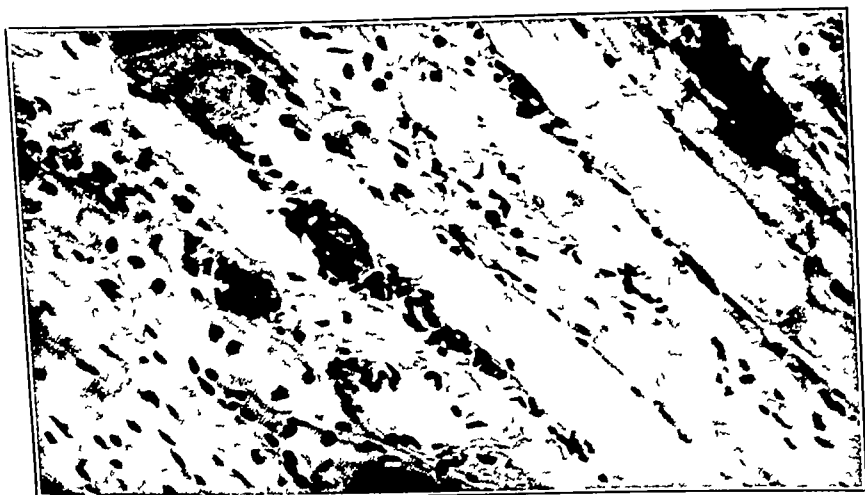


Fig 2—A photomicrograph of a section of the gastrocnemius muscle obtained at biopsy from Case 1. The variation in the size of the muscle fibers is less marked than in Fig 1 and the cross striations are more prominent. Areas of localized hyalinization of the muscle fibers are present. In scattered areas throughout the sections it appears that the sarcoplasm was being absorbed and the sarcolemma with pyknotic nuclei remains in a retracted state that gives the appearance at first glance of a collection of cells that may be mistaken for plasma cells or lymphocytes. Magnified 300 diameters.

upon the activeness of many of their patients, while Schmidt<sup>1</sup> says that sooner or later asthenia becomes the chief complaint.

Two patients with anemia of pregnancy entered the Indianapolis City Hos-

pital at approximately the same time with the diagnosis of toxemia of pregnancy and in both cases the asthenia was of such marked degree that it suggested myasthenia gravis. Burr and McCarthy,<sup>12</sup> in 1901, reported two cases of myasthenia gravis. In one case the symptoms of weakness and numbness began one year after an abortion progressed during the next year and terminated fatally two years after onset. At necropsy a three or four months' fetus was discovered in the uterus. Blood studies were not reported, however, it was noted on admission that the patient was somewhat pale. Hun, Blumer, and Streeter<sup>13</sup> collected from the literature nine cases of myasthenia gravis associated with pregnancy.

**CASE 1**—M. G., a white married female, aged thirty one years, entered the hospital in a semi stuporous condition. She had enjoyed good health until the present illness. She had had two normal pregnancies, the last one three years before. The last menstruation occurred three months before admission.

The present illness began seven weeks before admission with nausea and vomiting after each meal. Vertigo, headache, and blurring of vision developed. There was difficulty in focusing the eyes for any length of time. One week before admission weakness became so marked that she was unable to phonate and at times appeared irrational.

On physical examination the patient appeared toxic, was irrational and easily excited. There was a slow lateral nystagmus of both eyes with ptosis of the left eyelid and paralysis of the left occipitofrontal muscle. Dyspnea was marked while lying quietly, and the heart rate was 140 beats per minute. The axillary temperature was 99° and the blood pressure was 122/80. There was an enlargement of the uterus equivalent to four months' pregnancy. The liver and spleen were not enlarged. The muscles were flaccid and atrophied with marked coarse tremors and tenderness upon deep palpation. The knee jerks were absent. The urine examination revealed several white blood cells and a trace of albumin. A secondary anemia was present with R. B. C. 3.3 million, Hb 57 per cent, and 5.1 per cent reticulocytes. The W. B. C. was 12,000. The blood, nonprotein nitrogen, sugar, calcium, bilirubin, and Wassermann were normal. The spinal fluid was negative.

A diagnosis of toxemia of pregnancy was made and pregnancy was terminated one week after admission. The clinical condition remained unchanged during the next two months. During the third month in the hospital she was able to phonate, the pulse rate and temperature declined, and there was marked improvement in muscular weakness, but she continued to have irrational periods.

Electrical reactions of the muscles showed a decreased response and marked fatigability to intermittent faradic current. Galvanic current showed no evidence of peripheral nerve lesion.

A specimen of gastrocnemius muscle obtained at biopsy showed considerable atrophy of the muscle fibers without definite lymphohemorrhage as shown in Figs. 1 and 2.

The patient was discharged markedly improved after 132 days in the hospital.

**CASE 2**—E. I., a colored married female, aged twenty five years, entered the hospital in a stuporous condition. She had always been well until the present illness. She had one pregnancy that ended in stillbirth at nine months. The last menstruation was four months before admission.

The present illness began ten weeks before admission with morning nausea and roaring in the head. Vomiting and weakness developed and four days before entry she became drowsy with loss of orientation and coordination.

On physical examination the patient did not appear toxic. There was a lateral nystagmus of both eyes. The voice was weak and dyspnea was present while lying quietly. The heart rate was 100 beats per minute. Temperature 101°. The blood pressure was 95/33. There was an enlargement of the uterus equivalent to four months' pregnancy. The liver and spleen were not enlarged. The muscles of the extremities were flaccid. The knee jerks and biceps reflexes were absent while the other reflexes were normal.

The urine showed a trace of albumin and 50 W. B. C., 10 R. B. C., and an occasional hyaline cast per high power field. A primary type of anemia was present with R. B. C. 2.9 million, Hb 65 per cent, 6.8 per cent reticulocytes, and W. B. C. 8,800. The blood, nonprotein nitrogen, sugar, calcium, bilirubin, and Wassermann were normal. The spinal fluid was negative.

The diagnosis of toxemia of pregnancy was made and treatment instituted. Two months after entry the clinical condition remained unchanged. Gastric analysis showed free HCl present. Electrical reactions of the muscles showed marked fatigability to intermittent faradic current and no evidence of peripheral nerve lesion by galvanic current. A section of the gastrocnemius muscle revealed atrophy of the muscle fibers without lymphorrhagia as shown in Figs. 3 and 4.

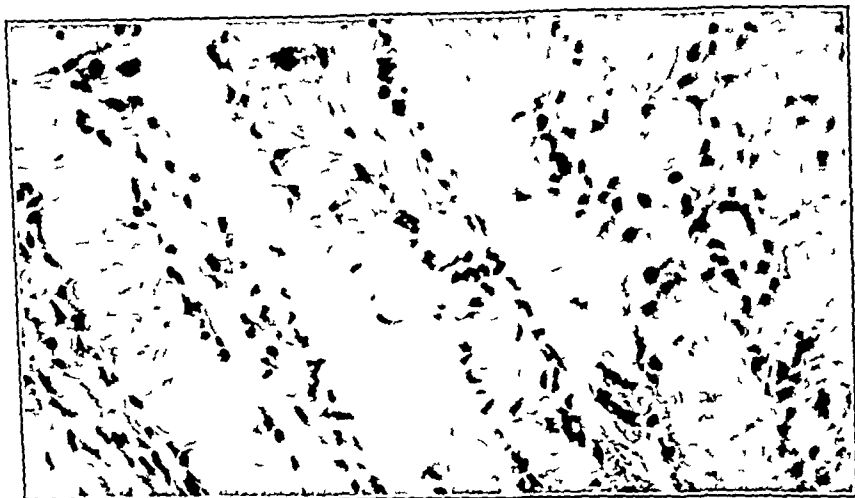


Fig. 3—A photomicrograph of a section of gastrocnemius muscle obtained at biopsy from Case 2. There is a marked variation in the size of the muscle fibers and the nuclei of the sarcolemma appear more prominent about the small fibers. The areas of retraction of the sarcolemma with the collection of its pyknotic nuclei as shown in Fig. 2 are less numerous in the sections of this case and are not shown in the illustrations. Magnified 200 diameters.

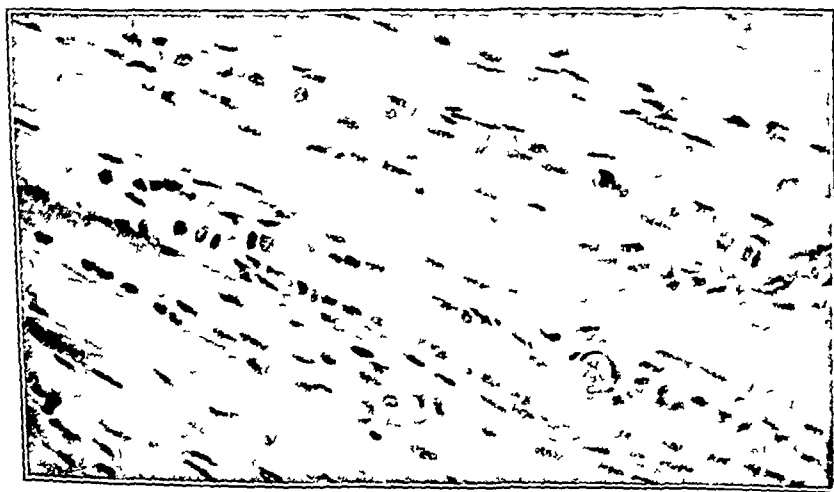


Fig. 4—A photomicrograph of a section of the gastrocnemius muscle from Case 2. The variation in size of the muscle fibers is less marked and the cross striations are present in some areas. The blood vessels appear normal without perivascular infiltration. Magnified 300 diameters.

There was steady improvement until discharge 113 days after admission.

Two months later she had a normal delivery. On the eighth day postpartum dysuria and fever developed. There was a sudden return of the previous symptoms and the patient reentered the hospital.

The blood showed R B C. 1.0 million, Hb. 21 per cent, reticulocytes 2.5 per cent, and W B C.

6,900 The other significant physical and laboratory findings were similar to the previous admission

Three vials daily of liver extract, No 343, were begun The reticulocyte and red blood cell

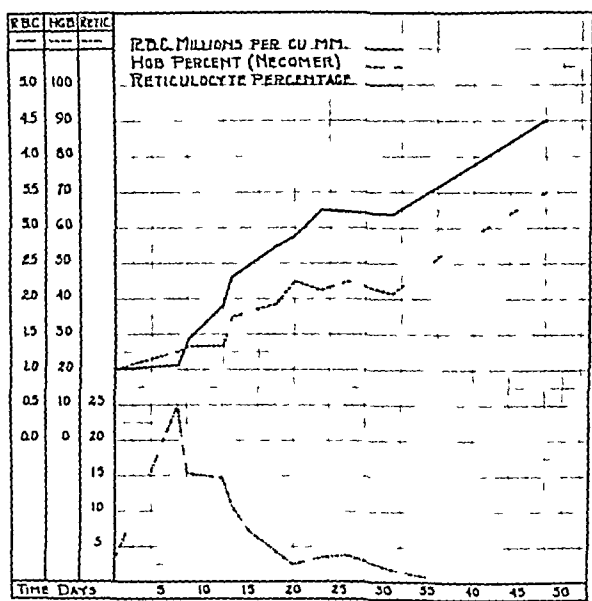


Fig 5 —(Case 2) Shows response of blood to 3 vials daily of liver extract No 343

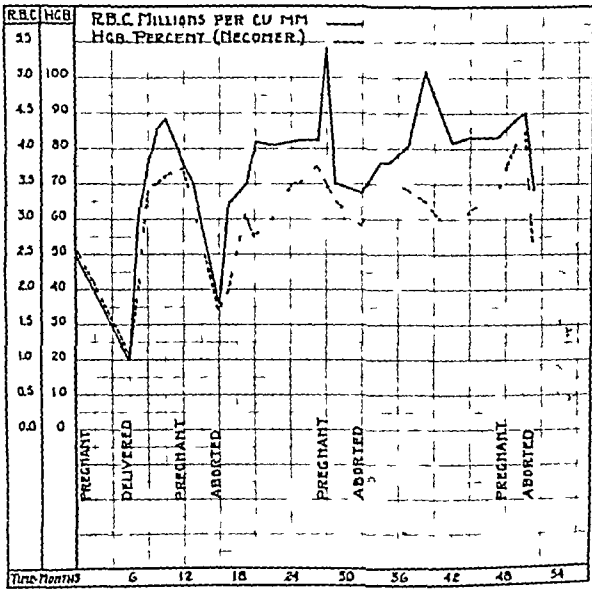


Fig 6 —(Case 2) Shows the course of the blood during four years observation with four pregnancies

response are shown in Fig 5 There was steady clinical improvement until discharge, seventy five days after admission

During the next three and one-half years there were three pregnancies, each terminated at four or five months Anemia was present during each of these pregnancies as shown in Fig 6

The previous symptoms were present during the first and last of these pregnancies but absent during the second.

Electrical reactions of the muscles during the last pregnancy were normal and a specimen of muscle obtained at the site of the previous biopsy showed no atrophy or lymphorrhagia.

Case 1 moved from the city soon after discharge and was not followed, while Case 2 was followed for four years. Both cases presented the typical clinical picture of myasthenia gravis with the myasthenic reaction but without the presence of lymphorrhagia in a skeletal muscle. The myasthenic reaction has been found in numerous conditions but lymphorrhagia in a skeletal muscle has been reported only in myasthenia gravis and in one case of amyotrophic lateral sclerosis, according to Keschner and Strauss,<sup>14</sup> and lymphorrhagia is found in only about 50 per cent of the cases of myasthenia gravis. In myasthenia gravis the presence of lymphorrhagia is not parallel to the severity of the involvement of a muscle, and has not always been demonstrated in muscles with marked involvement.

In Case 2 there was marked atrophy of the muscle fibers in the specimen removed at the first biopsy, while in the specimen obtained four years later from the same muscle and at the site of the previous biopsy, the histologic picture was normal.

The subsequent findings in Case 2 are in accord with the diagnosis of pernicious anemia of pregnancy, although Case 1 had a secondary type of anemia. The presence of a secondary anemia with the clinical picture of pernicious anemia of pregnancy has been reported in two cases by Evans. Wills and Mehta have seen patients with secondary anemia associated with pregnancy rapidly change to a primary type. The presence of fever in both cases is more in accord with this diagnosis than with the diagnosis of true myasthenia gravis.

The parallel progress of both patients even though pregnancy was terminated in Case 1 and not in Case 2 agrees with the statement of Schmidt that premature termination of pregnancy, induced or otherwise, did not have any influence upon the progress or improvement of the clinical condition of the patient.

The electrical reactions in pernicious anemia of pregnancy have not been reported in the literature. The pathologic findings were reviewed by Schmidt, and Balfour reported the findings in two cases, but the histologic findings in the skeletal muscles were not stated.

The presence of primary pernicious anemia in the negro is rare, only 30 cases have been reported in literature according to Mathews.<sup>15</sup> In Case 2, the anemia was of a primary type and occurred in a negro with free HCl present in the gastric contents. We have been unable to find any report in the literature of pernicious anemia of pregnancy in the negro.

In Case 2, the anemia and symptoms returned immediately after delivery and again in varying degrees during the three subsequent pregnancies, as shown in Fig 5. This is in agreement with the findings of Murdock, Evans, and McSwinnney. The premature termination of the three subsequent pregnancies further supports McSwinnney's findings that premature termination of pregnancy tends to occur.

The response of the blood, in Case 2, following the administration of liver extract, is shown in Fig 6. The efficiency of liver and liver extract upon the anemia in the pernicious anemia of pregnancy has been shown by Evans,<sup>9</sup> Peterson, Field and Morgan,<sup>17</sup> and others. However, the effect of liver extract upon the recurrence

of the symptoms in subsequent pregnancies and upon the tendency to premature termination of pregnancy has not been mentioned in the literature and further study is needed in this regard

#### SUMMARY

1 Two cases of anemia of pregnancy with the clinical findings and electrical reactions of myasthenia gravis are reported one patient having a secondary and the other a primary type of anemia

2 The primary type of anemia occurred in a negro patient, with free HCl present in the gastric contents and a typical response of the blood was obtained following the administration of liver extract, No 343

3 The case with a primary type of anemia has been followed for four years with three subsequent pregnancies all of which were accompanied by a relapse of the blood

4 The clinical findings and electrical responses of myasthenia gravis were absent in the negro patient when examined four years later

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## A CASE OF SARCOMA BOTRYOIDES<sup>\*</sup>

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**S**ARCOMA botryoides is the name applied to a comparatively rare polypoid tumor occurring within the cervix uteri. The unusual character of this tumor renders it a well defined pathologic entity. According to Wilms,<sup>1</sup> the sarcomas botryoides are true mixed tumors developed from misplaced embryonal mesodermic cells. Their early appearance and complex histologic structure indicate their origin to be probably the result of some embryogenic disturbance.



Fig 1—Thick edematous mucous membrane of bladder and long urethra

A review of the literature shows a marked discrepancy in the number of cases reported. McFarland<sup>2</sup> collected 34 cases, Veit<sup>3</sup> 40, Labhardt<sup>4</sup> 60, and K. Adler<sup>5</sup> could find only 41 authentic cases as late as 1928.

The case that I am reporting is that of a negro child, aged four years. She was admitted to the hospital August 23, 1931, from the out-patient department, with a diagnosis of gonorrheal vaginitis. In June, 1931, she had undergone a vaginal operation elsewhere, for the removal of a large tumor. This tumor had not been submitted for pathologic examination.

Examination revealed a profuse, foul, brownish-yellow vaginal discharge which, according to her mother, had been present for some time, and since the operation had become much worse. The mucous membrane of the vulva was dark reddish grey. The inguinal lymph nodes were slightly enlarged. There was con-

<sup>\*</sup>Received for publication April 3 1932.

siderable urinary retention. On admittance to the hospital the patient's temperature was 100° F, pulse 130, and respiration 28.

Aside from the above mentioned facts nothing of note was found on physical



Fig 2—Large thin walled uterus, sacculated upper third of vagina and lower two-thirds packed with polypoid masses of the growth.

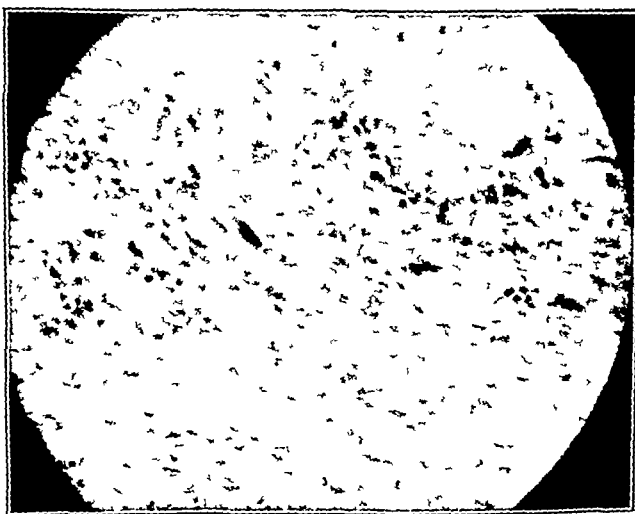


Fig 3—Section of the tumor showing cellular fibrous tissue and a few muscle fibers with large nuclei.

examination. Laboratory findings showed a leucocyte count of 31,000, with 84 per cent neutrophils. Urine examination was negative except for a few pus cells. Smears from the urethral and vaginal discharges showed a mixed infection consist-



ing chiefly of colon bacilli. Later there was a decided increase of pus cells in the urine.

It was necessary to catheterize the patient during her entire stay in the hospital. Catheterization was difficult and painful and, as it became progressively more so, on September 7, she was anesthetized and a retention catheter inserted. At this time examination showed a large smooth tumor very much like a distended bladder, which reached to the level of the umbilicus.

The treatment consisted of warm soda bicarbonate douches and potassium permanganate, 1:4000 dilution. The bladder was irrigated with boric acid. She was also given hot sitz baths and glucose and saline intravenously. She died September 11, 1931.

At postmortem examination there was found a marked pelvic peritonitis, hydronephrosis of the right kidney, and central necrosis of the liver. The uterus and bladder formed a large, lobulated, fluctuating mass that extended practically to the level of the umbilicus. The bladder was large and the mucous membrane was thick, polypoid, and very edematous. The urethra was considerably longer than normal, measuring 5 cm. The body of the uterus was the size of an adult's, and was filled with thick pea green foul purulent liquid. It had a granular brownish-red necrotic lining. The uterine wall was thin, averaging 5 mm in thickness. The vagina measured 13 by 10 cm, the upper 4 cm, forming a thin walled pocket similar to the uterus and in turn was filled with thick foul purulent liquid. The lower 9 cm of the vagina was distended with a large soft edematous papillary tumor that was attached chiefly to the posterior vaginal wall, the anterior wall being entirely free. The tumors varied considerably in size (from 5 mm to 5 cm). Many of the larger ones showed superficial areas of necrosis and hemorrhage. Microsections showed the tumor to be composed chiefly of loose cellular fibrous tissue, scattered through which there were a few smooth and striated muscle fibers. The more superficial portions were diffusely infiltrated with inflammatory cells. Sections from the less edematous areas of the tumor showed a few large cells that had large oval, slightly elongated granular hyperchromatic nuclei.

#### CONCLUSION

A case of sarcoma botryoides with recurrence and death two and one-half months after surgical removal is described.

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## PENETRABILITY OF TRANSPARENT MATERIALS FOR ULTRA-VIOLET RAYS\*

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IN THE course of experiments with ultra-violet ray irradiation of tubercle bacilli, it became necessary to know which material used as cover for cultures would be most penetrable for ultra-violet rays and thereby would permit the greatest bactericidal effect.

The following materials were tested by skin irradiation as well as culture irradiation experiments.

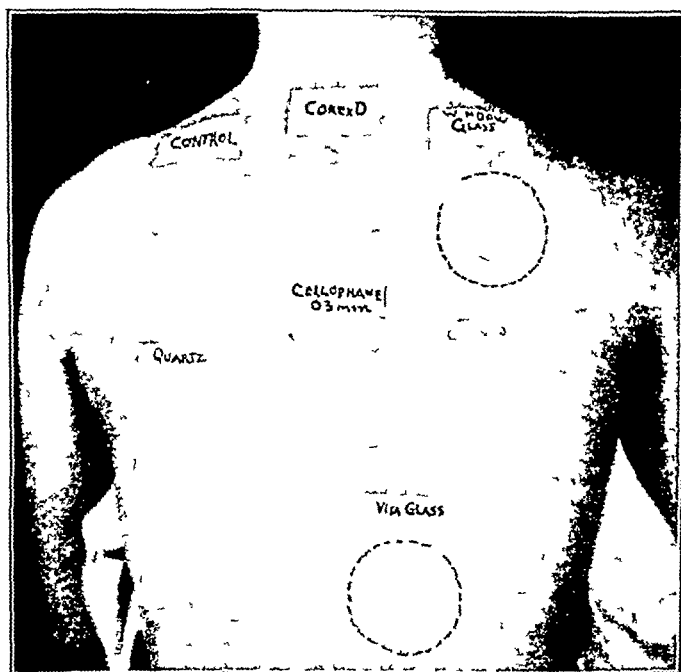


Fig. 1

Cellophane	0.03 mm thick (No. 450)
Cellophane	0.13 mm thick (No. 1800)
Vitaglass	3.0 mm
Window glass	3.5 mm
Quartz glass	2.0 mm
Corex D (Quartz glass)	2.0 mm
Uncovered control	

It seemed to the authors that the results illustrated in Figs. 1 and 2 are quite indicative of the comparative value of these various transparent materials for culture experiments.

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A mercury vapor lamp (Alpine Sunlight Hanovia Company) was used for the irradiations

Fig 1 skin irradiation experiment Irradiation time seven minutes, distance, 60 cm The areas encircled by dotted lines are those irradiated through ordinary window glass and Vitaglass respectively There was no erythema in either of these areas The Corex D showed slightly more erythema than the cellophane 0.13 mm

IRRADIATION TIME	CELOPHANE 0.03 mm (450)	CELOPHANE 0.13 mm (1800)	VITAGLASS	UNCOVERED CONTROL	WINDOW GLASS	QUARTZ GLASS	COREX D
10 min	+	+	+++	++	+++	++	++
15 min	+	+	+++	++	+++	++	++

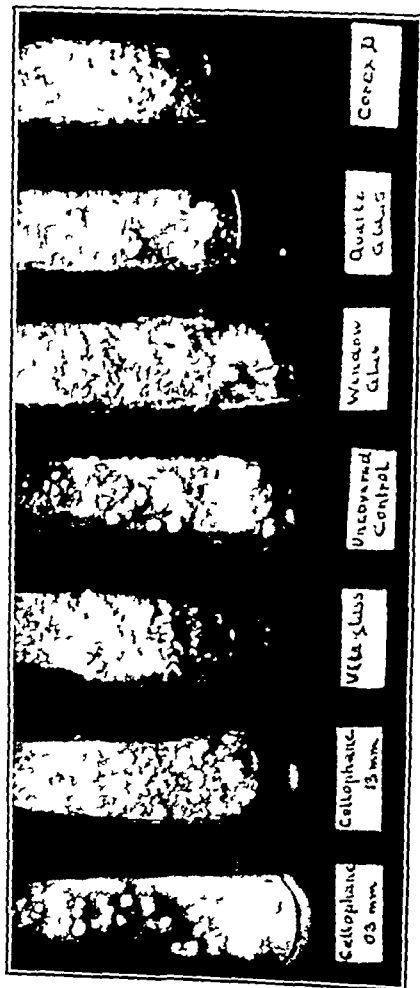


Fig 2

area, while the skin effect in the cellophane 0.03 area was equal to and even greater than that in the Quartz glass and uncovered control areas

Fig 2, culture irradiation experiments Irradiation time fifteen minutes, distance, 30 cm A suspension of tubercle bacilli of the virulent human strain H 119 was used Eight milligrams of organisms were distributed in 1 c.c. of sodium phosphate solution M/15 The irradiation was carried out on 2 1/2 mm deep layers of the suspension in Stender dishes Two parallel experiments with ten minute and

fifteen minute irradiation times respectively are recorded (The fifteen minute period tubes of the experiment with the 0.13 mm cellophane were lost and only the ten minute irradiation results recorded) Three culture tubes were planted for each irradiation period, that is, a total of 6 tubes was available for each transparent medium. The additional five minutes hardly effected any difference in the growth of the organisms. The recorded readings of the cultures were made four weeks after their implantation. The tabulation in Fig. 2 records the growth in each culture tube 0 indicating definite absence of colonies,  $\pm$ , the presence of a few colonies, +++, the growth of a maximum of colonies. Below each column in the figure, the photograph of a representative culture is shown. Only the window glass and Vitaglass cultures showed a maximum number of colonies in all tubes. With the 0.13 mm cellophane this was nearly so, while Correx D produced a slight but definite inhibition of growth. With 0.03 mm cellophane great inhibition was observed, in fact, as much as was present in the uncovered controls and in the cultures of bacilli irradiated through Quartz glass.

It will be noted that the findings by the culture experiments correspond well with those of the skin irradiation. Vitaglass, as well as window glass, stands out for failure to allow penetration of ultra-violet rays of erythema producing or bacteriocidal (tubercle bacilli) wave lengths, while thin cellophane of 0.03 mm (or less) strength is recognized as an excellent material for ultra-violet ray transmission. It is hardly inferior to pure Quartz glass and of sufficiently low cost to allow its generous use in laboratory work. We have been using it in culture work for some time with results equally as gratifying as those reported by Johnson\*. At the same time the experiment throws an interesting light on the value of the various transparent materials for general use in prophylactic and therapeutic irradiation.

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### BLOOD NONPROTEIN NITROGEN AND CREATININE IN NEPHRITIS AND PROSTATIC OBSTRUCTION†

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THE significance of increased concentration of creatinine in the blood has attracted considerable attention since it was first described in patients with uremia by Neubauer,<sup>1</sup> Folm and Denis,<sup>2</sup> and Myers and Fine.<sup>3</sup> Myers and Lough<sup>4</sup> stated that creatinine retention occurs, practically speaking, only in nephritis, and that values of 2.5 to 3 mg per 100 cc are presumptive evidence of renal disease, 3.5 mg indicating severe renal damage, values above 5 mg indicating a fatal termination, usually within two months. These conclusions were reaffirmed by Chace and Myers,<sup>5</sup> Myers and Kilham,<sup>6</sup> and Rabinowitch.<sup>7</sup> It was believed that the amount of increase of creatinine should be a more reliable index of the extent of renal functional damage than is that of urea or uric acid because of its endogenous origin and constant elimination under normal conditions. Myers, Fine and

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\*Johnson F. H. Cellophane Covers for Petri Dishes for Keeping Out Contaminations and Studying the Effects of Ultra-Violet Light. *Science* 73: 680, 1931.

†From the Laboratory of Biochemistry and the Department of Medicine, Jefferson Hospital.  
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Lough<sup>8</sup> observed that in early nephritis the blood uric acid was increased, the blood urea but slightly increased and the blood creatinine remained within normal limits, producing a staircase effect. This was interpreted as evidence that, in nephritis, elimination of uric acid is impaired first, that of urea later and that of creatinine last.

Subsequent investigations have in general failed to substantiate this interpretation of the mechanism underlying the relatively late rise in the concentration of creatinine in the blood in chronic nephritis. Watanabe<sup>9</sup> states that although creatinine appears to be excreted slightly more readily than urea under normal conditions, there would appear to be comparatively little practical difference in the ability of the kidney to eliminate these two waste products. This condition is supported by the recent work of Cope<sup>10</sup> who showed, by a comparison of blood urea and creatinine clearance studies, that in the nephritic subject the impairment of creatinine excretion approximately parallels that of urea excretion, the normal ratio between the two being roughly maintained for all degrees of renal damage. Berglund<sup>11</sup> likewise reported uniformity of retention of urea and creatinine in chronic nephritis. However, the serious prognostic significance of elevated blood creatinine values in chronic nephritis has been amply confirmed. This is not true of acute nephritis nor of acute exacerbations of chronic nephritis, obviously because of the possible transitory nature of the acute lesion in such cases. For example, Campbell<sup>12</sup> reported a case of acute bichloride nephrosis with recovery in which the blood creatinine had risen to 12.5 mg per 100 c.c. Gatewood and Byfield,<sup>13</sup> reported a similar case with a blood creatinine concentration of 13.1 mg per cent. Griffith and Cantarow<sup>14</sup> observed a patient with chronic glomerulonephritis in whom, during a period of acute exacerbation, the blood creatinine rose to 15 mg per 100 c.c., subsequently falling to 1.4 mg.

The statement is frequently made that most of the extrarenal conditions which are associated with an increase in other nonprotein nitrogenous constituents of the blood have little or no effect on the creatinine concentration. Cases of pyloric or acute intestinal obstruction are, however, not infrequently encountered, in which rather marked grades of creatinine retention exist, as in two instances reported by Griffith and Cantarow.<sup>14</sup> From a practical standpoint, perhaps the most important group of extrarenal conditions associated with blood nitrogen retention is represented by obstructive lesions of the lower urinary tract, of which prostatic hypertrophy is the most common. Patch and Rabinowitch<sup>15</sup> reported 5000 observations of the relationship between the blood urea and creatinine concentrations in patients with renal disease alone, with obstructive extrarenal lesions alone, and with obstructive lesions complicated by organic disease. These observers found that whereas in nephritis there was a distinct parallelism between blood urea and creatinine values, in uncomplicated obstructive lesions the blood urea concentration was increased out of all proportion to that of creatinine. In those cases of urinary obstruction showing evidence of superimposed renal damage (casts) the findings were similar to those observed in nephritis. These observations are so different from our own experience that this study was undertaken for the purpose of comparing the relative degree of creatinine retention in nephritis with nitrogen retention and in prostatic obstruction with nitrogen retention.

## PRESLAV INVESTIGATION

The present series consists of 112 patients with nephritis (acute and chronic glomerulonephritis and nephrosclerosis) and 80 patients with urinary obstruction due to prostatic hypertrophy. Simultaneous blood total nonprotein nitrogen and creatinine determinations were carried out in each instance.

In the group of patients with nephritis, the blood nonprotein nitrogen concentration ranged from 40 to 289 mg and the creatinine from 1.22 to 23.47 mg per 100 c.c. In those with prostatic hypertrophy, the blood nonprotein nitrogen varied from 40 to 202.7 mg and the creatinine from 1.4 to 15.82. The detailed findings are presented in Tables I and II. Tables III and IV, taken from the data of Patch and Rabinowitch,<sup>1</sup> are inserted for purposes of comparison. In comparing these data it must be realized that our figures represent total nonprotein nitrogen whereas those of Patch and Rabinowitch represent urea nitrogen. It may be noted that the relative degree of creatinine retention in our series of patients with nephritis is distinctly greater than was present in the cases reported by these observers. This discrepancy is difficult to explain. It may be that their cases of

TABLE I  
NEPHRITIS

CASES	NONPROTEIN NITROGEN*	AVERAGE*	CREATININE*	AVERAGE*
24	40.50	45.82	1.22-2.34	1.87
15	50.1-60	54.0	1.52-3.26	2.07
9	60.1-70	65.36	1.22-9.44	3.71
5	70.1-80	73.60	2.06-4.02	2.63
7	80.1-90	87.39	1.78-8.20	5.82
2	90.1-100	95.25	2.74-2.76	2.75
9	100.1-110	103.77	3.28-8.10	5.64
8	110.1-120	116.67	3.22-10.90	6.99
6	120.1-130	122.80	3.42-10.10	7.45
12	130.1-170	146.8	3.34-18.00	10.50
4	170.1-200	187.39	11.52-17.40	14.02
11	200.1-289	252.21	9.64-23.47	15.07

\*All values expressed in milligrams per 100 c.c.

TABLE II  
PROSTATIC HYPERTROPHY

CASES	NONPROTEIN NITROGEN*	AVERAGE*	CREATININE*	AVERAGE*
23	40.50	43.72	1.40-2.58	1.96
14	50.1-60	55.48	1.88-3.06	2.34
9	60.1-70	64.89	1.97-6.00	3.23
7	70.1-80	75.11	2.06-5.16	3.15
3	80.1-90	84.52	3.44-5.34	4.54
4	90.1-100	94.50	2.36-4.50	3.47
2	100.1-110	105.86	4.50-11.50	8.00
4	110.1-120	115.92	6.90-10.58	8.68
3	120.1-130	125.58	7.10-9.44	8.21
6	130.1-170	144.68	4.86-15.82	9.83
4	170.1-200	184.11	8.35-14.30	11.19
1	202.70	202.70	11.76	11.76

\*All values expressed in milligrams per 100 c.c.

TABLE III  
BLOOD UREA NITROGEN AND CREATININE IN KIDNEY DISEASE  
(AFTER PATCH AND RABINOWITCH)

UREA NITROGEN*	AVERAGE CREATININE*
20-50	1.2-1.89
51-90	2.21-2.93
91-120	3.10-3.71
121-170	4.28-5.86
171-200	5.86-7.87
201	9.30

\*All values expressed in milligrams per 100 c c

TABLE IV  
UROLOGIC CONDITIONS  
(PATCH AND RABINOWITCH)

	UREA NITROGEN*	CREATININE*
Prostatism	168	2.4
Prostatism	230	2.43
Urethral Stricture	168	2.14
Carcinoma of Bladder	105	1.57

\*All values expressed in milligrams per 100 c c

"kidney disease" included conditions other than nephritis. In our experience, creatinine values below 2 mg per cent are not commonly observed in association with urea nitrogen values as high as 50 mg in nephritis.

The difference is even more striking in the group of individuals with urinary obstruction due to prostatic hypertrophy. In this series the degree of creatinine retention was essentially the same as in the patients with nephritis. In fact, with total nonprotein nitrogen values below 130 mg per 100 c c, the blood creatinine was somewhat higher in the prostatic than in the nephritic group, in the former it rose on an average of 0.0513 mg per cent for every milligram rise of nonprotein nitrogen above 40 mg, the corresponding rise in the latter group being 0.0452 mg. As the blood nonprotein nitrogen level rose above 130 mg per 100 c c, the creatinine values became more nearly equal, the degree of elevation in the prostatic group being 0.0695 mg per cent for each milligram increase in nonprotein nitrogen, and in the nephritic, 0.0698 mg per cent. No attempt was made in the present study to exclude from the prostatic group those individuals with superimposed renal lesions, a fact which might serve to explain these findings. However, the absence of serious renal damage in many cases with marked azotemia is evidenced by the fact that in 6 of the 11 cases of prostatic hypertrophy with nonprotein nitrogen values above 130 mg and creatinine values ranging from 8.25 to 15.82 mg per cent, both fell to within normal limits following relief of the obstruction to the outflow of urine.

#### DISCUSSION

In our experience purely obstructive urinary tract lesions cannot be differentiated from renal disease on the basis of the degree of elevation of blood creatinine

In fact, in some instances, particularly in those cases with nonprotein nitrogen values below 130 mg per 100 cc, the rise in blood creatinine was slightly greater in the prostatic than in the nephritic group. A similar observation has previously been made in conditions such as acute intestinal obstruction, associated with pre-renal deviation of water without demonstrable organic renal disease.

The discrepancy between our findings and those reported by Patch and Rabinowitch<sup>15</sup> is difficult to explain. These authors suggested that since the observed difference in creatinine values in nephritis and urinary obstruction could not be explained on the basis of different rates of excretion, the greater part of what is regarded as creatinine in nephritis is probably not creatinine but rather some unknown substance that gives the Jaffe reaction which, as is well known, is not specific for creatinine. In support of this belief they state that the observation of a positive diazo reaction in cases of advanced nephritis indicates the presence of some undetermined substance not present in the blood serum of individuals with urinary obstruction with a similar degree of urea retention. It is implied that this substance is perhaps responsible for the apparent difference in the degree of creatinine retention in the two groups of conditions. One must admit the doubtful identity of the substance or substances which are responsible for the reaction commonly attributed to creatinine in the blood. In fact, Behre and Benedict<sup>16</sup> go so far as to question the existence of creatinine in the blood. However, the data presented here do not support the hypothesis of the accumulation, in nephritis exclusively, of some specific substance giving the Jaffe reaction.

#### SUMMARY

1 Simultaneous determinations of nonprotein nitrogen and creatinine were made in the blood of 112 patients with nephritis and 80 patients with urinary obstruction due to prostatic hypertrophy in all of whom nitrogen retention was present.

2 It was found that, contrary to some previously reported observations, the degree of creatinine retention was practically the same in both groups of patients. With total nonprotein values below 130 mg per 100 cc the average increase in blood creatinine was slightly greater in the obstructive than in the nephritic group.

3 It appears that no distinction can be made between purely obstructive urinary lesions and actual renal disease on the basis of the relative degree of blood creatinine elevation.

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## STUDIES ON CALCIUM LEVULINATE WITH SPECIAL REFERENCE TO THE INFLUENCE ON EDEMA\*

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**I**N SOME studies of calcium metabolism the question was raised not infrequently as to the effectiveness of various salts in elevating the calcium content of the blood<sup>1</sup>. Of interest were comparisons between calcium lactate and calcium gluconate in tuberculous patients. A satisfactory elevation (1 to 2.5 mg per 100 c.c. of blood) above the "resting level" was noted following the administration of 3 or 5 gm. of calcium lactate on an empty stomach but this was accompanied usually by gastrointestinal manifestations (loss of appetite and nausea), especially if administration was continued (1 or 2 times daily) for three or four days. A smaller dose (0.5 to 2 gm.) had no constant influence on the blood calcium level even in prolonged administration (2 or 3 times daily), and in certain instances gastrointestinal symptoms occurred. In the use of the salt intravenously (5 or 10 c.c. of a 10 per cent solution) elevations from 1 to 2.5 mg per 100 c.c. of blood (above the resting level) were noted. The injections were not entirely free from untoward effects even when the greatest care was used in administration. Of special concern was the frequency of local tissue reaction and vessel thrombosis. The subcutaneous and intramuscular injections were prohibited because of severe pain, inflammation and the danger of necrosis.

The variations in blood calcium following calcium gluconate administration (oral and intravenous) were similar to those in the use of calcium lactate except

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that relatively larger doses were required in order to obtain corresponding elevations. The untoward effects were less frequent and disturbing than in calcium lactate administration, except that diarrhea was not uncommon in prolonged use. In a few instances unexplained general reactions (chills and slight elevation of temperature) occurred during or after the intravenous injections. The subcutaneous and intramuscular injections were essentially free from untoward effects, but hypercalcemia was not striking except when more than 8 c.c. of a 10 per cent solution were used. Further experiences suggested criticisms. These were (1) low solubility in cold water (usually incomplete except in warm water), (2) the marked tendency to precipitation of 10 per cent solutions in ampoules (3) the relatively low calcium content per gram of calcium compound (calcium gluconate contains 9.3 per cent Ca, calcium lactate 13.03 per cent Ca). The possibility that incomplete absorption (oral administration) was due in part to low solubility and that precipitation was responsible for the general reactions (intravenously) suggested that a study of other calcium salts would be of interest.

In a search of the literature it was found that Weintraub<sup>1</sup> attempted in 1894 to throw light on the relationship between levulinic acid and the occurrence of acetonaemia. He used calcium and sodium levulinate experimentally and reported no toxic effects. In 1919 Frankel<sup>2</sup> considered that the salts of levulinic acid were toxic and referred to the studies of Weintraub as evidence, misinterpreting apparently this early work. Since the salt had certain theoretical possibilities, preparation was undertaken at the Chest Department of the Jefferson Hospital in 1930. In October of the same year a pure, neutral, soluble (30 per cent in cold water), stable (30 per cent in ampoules) compound containing 13 per cent of calcium was obtained.

#### PRELIMINARY OBSERVATIONS

*Oral Administration*—Calcium levulinate was administered to animals as follows. Thirty female albino rats, each weighing 120 gm. were fed an average maintenance diet for fifteen days. The animals were apparently in good condition at the end of this period. A further series was selected for oral administration. The animals were caged individually and continued on routine care except that the food pans were removed each evening. In the morning a small amount of ground whole wheat with 0.65 gm. of calcium levulinate was fed to each animal. After consuming this ration the control diet with water was then fed ad libitum. None of the animals showed diarrhea, weakness, or loss of weight.

*Subcutaneous Injections*—A sterile 10 per cent solution of calcium levulinate was prepared by intermittent sterilization. This was injected in varying doses into the abdominal wall. Four rats each received 1 c.c., four 2 c.c. and four 3 c.c. every fifth day for four injections. There were no untoward effects and the solution was apparently well absorbed.

*Intravenous Administration*—Injections of a 5 per cent sterile solution were made slowly into the coccygeal veins of seven rats. These injections were made on alternate days until five doses were administered. The doses varied from 1 c.c. to 3 c.c., in one animal the dose was 5 c.c. No harmful effects were noted except following the administration of 5 c.c. when the animal exhibited signs of collapse. Recovery occurred rapidly and subsequently four doses of 3 c.c. were injected without causing reaction.

## FURTHER OBSERVATIONS

The compound was administered to far advanced tuberculous patients (service of Dr. N. Blumberg, Philadelphia General Hospital) orally (1 to 2 gm. dissolved in 50 c.c. of water once or twice daily) and subcutaneously (3 to 5 c.c. of a 10 per cent solution two times weekly for four doses). The longest period of oral administration was three weeks, the average being eleven days. Three patients experienced transient loss of appetite and nausea on the third day and administration was discontinued. The subcutaneous injections (seven patients) were followed by pain and discomfort at the site of administration, which lasted from a few hours up to three days, but there was no evidence of necrosis.

Studies to determine the immediate effects of calcium levulinate were undertaken in a series of 14 tuberculous patients at the Jefferson Hospital. The dose varied between 5 and 10 c.c. of a 14 per cent solution (three times weekly). Four patients each received 7 injections and the rest four injections. Clinical data on local and general reactions and variations in pulse rate, respiration and temperature were obtained before and at intervals for several hours after the injections. These showed no characteristic change except transient reduction in the respiratory rate, dryness of the throat and a sensation of warmth throughout the body during and immediately following administration. These manifestations were similar to those noted in the use of parathyroid hormone.<sup>1</sup> Slight soreness and inflammatory reactions lasting for two or three days appeared at the site of injection in five patients. These were due to extravasation of the salt into the tissues. Blood calcium determinations made at intervals following oral and subcutaneous administration showed no striking or characteristic change. However a sharp elevation of blood calcium (1.5 to 4 mg. per 100 c.c. of blood) above the "resting level" usually followed the intravenous injections. The "peak" occurred from three to ten minutes after administration and a gradual return to normal occurred within three hours. Definite reduction (one to three minutes) in the clotting time of the blood was noted in six patients receiving the salt intravenously. The maximum effect was noted within thirty minutes after administration.

An interesting clinical effect occurred in three patients with marked edema of the legs and abdominal wall. In the first case more than 500 c.c. of fluid exuded from a bed sore within two hours following injection of 8 c.c. of a 14 per cent solution and subsequent accumulations responded in a similar manner. In the second case with urinary output of less than 250 c.c. daily there was marked diuresis and reduction of edema within a few hours after administration. The condition returned in these patients and similar effects were noted after further injections. The third case with marked displacement of the heart due to pulmonary fibrosis and with swelling of the legs, abdomen and hands, experienced no effect on edema following a period of digitalization. He received 2 injections of calcium levulinate daily for three days and striking reduction of swelling occurred.

Since the immediate effects of calcium levulinate were nontoxic, oral, intravenous and intramuscular administration\* was undertaken in 26 patients with far advanced pulmonary tuberculosis to determine the effects of prolonged administration. Possible influences on tuberculous processes were not studied. The powder

\*The calcium levulinate (cavulin) was manufactured by Smith, Kline and French Laboratories.

for use orally was flavored with lime and mixed with lactose. The latter was employed according to the studies of Beigelm<sup>5</sup> who found that absorption of calcium carbonate was increased in an acid medium. The solutions for the injections contained from 3 to 14 per cent calcium levulinate and the dosage varied from 1 to 10 c c. The oral administrations (2 gm daily) were continued in 8 patients for six weeks. The intramuscular injections (2 injections weekly) were given to 7 patients for three weeks and the intravenous injections (1 to 4 weekly) to 11 patients up to twelve weeks. The greatest number of intravenous injections was 43 in one patient sixty-three years of age. The immediate symptomatic effects were the same as previously noted, except that patients receiving prolonged intravenous administrations experienced transiently, an increase of strength and decreased expectoration. These lasted usually for a few hours after each injection.

Blood calcium determinations were made at intervals during treatment. So far as determined there was no evidence suggesting that the prolonged oral and subcutaneous administrations maintained a constant elevation of the blood calcium of more than 1 mg per 100 c c of blood. In the majority of instances no definite change was noted. In the intravenous administrations transient elevations followed the injections.

It appears from the present investigation that calcium levulinate is essentially nontoxic and may be considered for trial in conditions in which calcium therapy is indicated. Certain features suggest definite advantages over other calcium salts. Of special interest is the high degree of solubility and stability in solution as compared with calcium gluconate and the relative freedom from severe local reactions which are not uncommon when extravasation occurs in calcium lactate administration. As in previous experiences with calcium salts administered orally to tuberculous patients in toleration doses, the effect of calcium levulinate on the resting blood calcium level was not striking. Incidentally this raises questions as to the rate and degree of absorption and mobilization of calcium following various methods of administration. These are pertinent in considering the large reservoir of blood and tissue fluids which receive a comparatively small dosage and because excretion under certain circumstances may almost parallel absorption. It would appear in general that intravenous administration should be employed when a sharp elevation of blood calcium is desired. In cases with mild calcium deficiency the simple administration of foods rich in calcium values (800 c c of milk contain more calcium than 13 gm of calcium gluconate) and the use of sunlight, cod liver oil, and viosterol should be effective. Mobilization may be increased by injections of parathyroid hormone.

Since this investigation was undertaken, Bennett<sup>6</sup> reported the use subcutaneously of a 3 per cent solution of calcium levulinate and calcium gluconate in tetany. The substance was rapidly absorbed without untoward effect and arrest of the tetany was secured. Geville and Dodds<sup>6</sup> used calcium levulinate intravenously (20 c c of a 10 per cent solution) in jaundiced patients without untoward effect.

#### CONCLUSIONS

- 1 Calcium levulinate contains practically the same amount of calcium as calcium lactate (about 40 per cent more than calcium gluconate), is soluble and stable in 30 per cent solutions.

2 In animal and clinical investigations (tuberculous patients), calcium levulinate was nontoxic and effective intravenously in increasing the calcium content of the blood above the normal level. A reduction in the clotting time of the blood occurred in a certain instance.

3 The immediate and striking influence on edema in three patients is noted. Symptomatic manifestations such as dryness in the throat, sensation of warmth, and decreased expectoration suggested "calcium effect."

We are indebted to Dr. W. L. Haight and Mr. R. J. Titherington for valuable assistance in this study.

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# LABORATORY METHODS

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## DEHYDRATED EGG MEDIA FOR ANAEROBIC CULTIVATION AND DIFFERENTIATION<sup>3</sup>

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EGG media have long been utilized for the cultivation of bacteria, and a variety of formulas have been developed according to the peculiar individual requirements, such as the Dorsett Petrioff Goldberger and Robertson media

Kahn<sup>1</sup> has utilized coagulated egg albumen cubes and a modification of the Robertson alkaline egg in the identification of a group of single-cell strains of anaerobes. We have followed these procedures but due to the time required for preparation, and loss from blowing of plugs a study was made to develop a satisfactory dehydrated substitute

Through the cooperation of the Difco Laboratories three forms of dehydrated preparations were made available, respectively whole egg, egg yolk, and egg albumen, each ground to a fine dry powder. The latter two were carefully separated to represent only the yolk or the albumen

Each of these may be prepared by placing approximately 0.5 gm. in a dry tube, preferably 8 by  $\frac{5}{8}$  inches, to which is added 10 mls. of distilled water or nutrient broth. A slight stirring brings the powder into suspension after which the tubes may be autoclaved by the usual process without tendency to blow, or even to wet the plugs. The medium thus prepared has a reaction very close to  $P_H$  7.2, which Kahn and others regard most satisfactory, and presents a loosely flocculent column of solids some 3 cm. deep with about 2 cm. of fluid above it.

These tubes may then be inoculated deeply by loop or preferably by pipette, with anaerobes from deep brain stock cultures, or from stock cultures in the whole egg medium which serves as an excellent medium for maintaining cultures over long periods. No vaseline or other seal is required, which is a great advantage in anaerobic cultivation, although it desired to determine the amount of gas formed the "vas-par" seal of Hall may be utilized.

The entire procedure is less tedious and more cleanly than that involved in the preparation of deep brain and similar media although it is not to be inferred that this disparages the many valuable features of the latter. The fact that this egg medium may receive routine autoclaving, with resultant insured sterility and no loss from blowing, considerably reduces labor and wastage.

In these egg media the various anaerobes produce characteristic reactions, only a few of which will be outlined below. These reactions consist of varying degrees of proteolysis, color changes, consistency of medium and gas formation.

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<sup>3</sup>From the Department of Bacteriology, West Virginia University Medical School  
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<sup>1</sup>Kahn, M. C. Pub. Cornell Univ. Med. Coll. 17: 155, 1919-1922

In addition to these reactions, the use of reduced iron (J. T. Baker Iron Powder Reduced by Hydrogen), as suggested by Hastings and McCoy at the Baltimore meeting of the Society of American Bacteriologists, both facilitates anaerobic growth and intensively accentuates blackening by the proteolytic organisms. For this purpose as little as 0.125 per cent sharply blackens with such organisms as *Cl. sporogenes* and *Cl. putrificum*. Larger amounts, up to 0.5 per cent seem to increase the growth and, of course, also the degree of blackening. Tubes inoculated with nonproteolytic organisms fail to show blackening and instead become reddish-brown, particularly near the surface, presumably with the formation of ferric oxide.

Lead acetate may also be incorporated to detect  $H_2S$ . Its toxicity, however, limits its use to a maximum of 0.2 per cent concentration, while 0.05 per cent produces an intense blackening with *Cl. sporogenes*, *Cl. tyrosinogenes*, and others.

As noted above, either distilled water or broth may be used as the diluent. With the latter (Difco Tryptone Broth) the physical state is more flocculent and the growth rate is considerably accentuated, due to the accessory food substances in the medium. Thus *Cl. tetani* in egg albumen with broth shows gas and bacterial turbidity within twenty-four hours, while with distilled water only the slightest evidence of growth appears within ten days.

Since the purpose of this report is primarily to describe the medium and its generally useful features, the reactions of only three representative organisms will be included.

1 *Cl. sporogenes*, an *Actively Proteolytic Type*—Whole egg in tryptone broth, active growth at twenty-four hours, decomposition evident, with 1 cm. of gas under "vas-par" seal, bright yellow initial color changing to a dirty gray-yellow. Prolonged incubation gives about 2.5 cm. of gas, and leaves about 0.2 cm. of grayish amorphous deposit after forty days. Odor is extremely foul. Sporulation is abundant at forty-eight hours. If 0.5 per cent reduced iron or 0.05 per cent lead acetate is incorporated the fluid becomes very black, with a black sediment.

Whole egg in distilled water, changes as noted above, but much less active. Blackening of iron is approximately the same.

Egg yolk, reactions scarcely distinguishable from those in the whole egg. Growth and gas formation seem even to be accelerated, and the amount of residual sediment at forty days is almost nil.

Egg albumen in tryptone broth, active growth at twenty-four hours, with evident decomposition as shown by slight translucence of the solids. About 0.5 cm. of gas at twenty-four hours, with a maximum of about 1.5 cm. Albumen completely decomposed at ten days, leaving only a slight grayish deposit. If iron or lead acetate are added blackening is evident at twenty-four hours, and increases to a maximum at three days.

Egg albumen in distilled water, no evidence of growth during the first twenty-four hours, but marked at forty-eight hours, and then generally following the character of the reactions in broth.

*Cl. putrificum*, *Cl. botulinum* and *Cl. tyrosinogenes* fall in this class, though in general much less active. *Cl. tyrosinogenes* produces an abundance of tyrosin crystals in all forms of the egg, but much more abundantly when tryptone broth is used as the diluent.

2 *Cl tetani*, a *Feebly Proteolytic Type*—Whole egg in tryptone broth, growth and gas delayed until forty-eight hours, bright yellow changes to dirty grayish-yellow at about ten days. Under "vas-pai" seal forms a maximum of about 1 cm of gas. Further reaction consists solely of a fractional separation of an upper layer of dirty gray flocculent material over a lower mass of yellow yolk. The odor in this medium is only moderately fetid. The initial mass of solids is reduced by only about  $\frac{1}{4}$  its volume in forty days. Sporulation is abundant at five days.

Whole egg in distilled water, growth greatly delayed and very inactive. Evidently egg alone is not well suited to the nutritional requirements.

Egg yolk in tryptone broth, as above, with practically no change in character or volume.

Egg yolk in distilled water, growth greatly delayed, with practically no change other than slight graying. Frequently transfers fail to show any evidence of growth.

Egg albumen in tryptone broth, no evidence of growth usually until fourth or fifth day, when gas appears. Very slow proteolysis occurs, and in presence of 0.5 per cent iron, beginning at about ten days, a mottled blackening appears in the whitish medium, increasing to maximum at about thirty days. Proteolysis is only partial at forty days.

Egg albumen in distilled water, usually no growth nor perceptible change in medium. Evidently the organism cannot successfully initiate growth in solely an albuminous medium.

*Cl edematiens* and *Cl edematis-maligni* have these same general cultural reactions.

3 *Cl welchii*, a *Nonproteolytic Type*—In all forms of egg, with broth or distilled water, produce active growth with abundant gas in twenty-four hours. The solids are raised to the top with the emeshed gas bubbles. No change in color or consistency occurs. No odor is observed. Sporulation is very slow, in fact of 4 strains tested spores have been observed in only 2 tested up to ten days. Microscopically evidence of sporulation is uncommon within thirty days.

All forms of the egg medium remain unchanged in color, even when iron or lead acetate are included.

No other anaerobe which we have studied shows such reactions.

#### SUMMARY

A series of three forms of dehydrated, powdered egg preparations are described. When prepared in tryptone broth, the whole egg presents an easily prepared medium very suitable for maintenance of stock cultures of the common sporulating anaerobes.

All forms, especially when combined with reduced iron in suitable concentration, show characteristic proteolytic changes which suggest useful application to the identification of species.

Characteristic reactions of three representative species are described.



# A ROUTINE CLINICAL EXAMINATION FOR TUBERCLE BACILLI IN MICROSCOPIC NEGATIVE SPUTUMS BY VARIOUS CULTURE METHODS\*

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THE tubercle bacillus grown in the incubator requires certain fundamental conditions approaching that of body environment. These must be maintained over a considerable period of time in order that small numbers of bacilli may multiply sufficiently to be discerned by the usual means of microscopic or macroscopic detection. In an earlier report<sup>1</sup> it was shown that to readily detect tubercle bacilli in smears of sputum and in sections it required at least about 100,000 bacilli per cubic centimeter. It was also shown that the guinea pig<sup>2</sup> was a reliable delicate test for small numbers (from 10 to 100) of human and bovine tubercle bacilli and that culture methods could hardly be expected to attain more than equal efficiency. This, however, was achieved by preliminary treatment with an equal volume of 6 per cent sulphuric acid or better, a 5 per cent oxalic acid solution<sup>3</sup> to destroy undesirable contaminants and then planting the washed residue on a satisfactory sterile nutrient medium, the crystal violet potato cylinder medium. The conditions of the medium including its moisture, neutrality, isotonicity, sterility, and general physical composition all play a part in maintaining conditions suitable for the proliferation of small numbers of bacilli. In earlier studies<sup>4</sup> it was shown that a medium (such as glycerol agar or Long's synthetic medium) might be well suited for supporting the growth of heavy plantings of tubercle bacilli, such as are obtained from sputums positive on microscopic examination, and that they might even appear to grow on these mediums more luxuriantly and more rapidly in heavy plantings than on good nutrient mediums. Poor nutrient mediums made of soup stocks or simple chemical salts, or good nutrients vitiated by dilution with inert substances like agar, were entirely unsuited for supporting the growth of small numbers of bacilli such as are found in negative specimens of smears or sections, using microscopic examination as the criterion.

As a result of earlier studies on the isolation of tubercle bacilli for diagnostic purposes a sulphuric acid or oxalic acid crystal-violet glycerol water potato culture method was described. The procedure consisted briefly, of destroying the contaminants with an equal volume of 6 per cent sulphuric acid or 5 per cent oxalic acid, mixing well with the suspected specimen and incubating for thirty minutes at 37° C. The material was then diluted with ten volumes of sterile 0.9 per cent saline solution to remove the acid and the residue after centrifuging was planted on the neutral potato medium. Since these experiments were performed and the method recommended, it has been found that the saline washing does not suffice to remove the acid from the tissue residue and that the material when planted may still register a low  $P_H$  (2.4), an acidity which may possibly persist in the planted

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specimen for some time after incubation is begun.<sup>5</sup> It was also found that a  $P_H$  below 4 would destroy the bacilli within a few days at incubator temperature. For this reason it appeared desirable to study the effect of chemical neutralization as opposed to washing alone for suspected sputum specimens.

It is obvious that only empiric material, "suspected sputums," was available for such a study since seeded control sputums could hardly be used without introducing the factor of bacilli already present in the sputums. It is our experience also that results obtained with studies pursued with sputums positive in microscopic smears are of no value in studying methods for isolation, or for studying the value of nutrients for small numbers of bacilli. The striking results from the study with sputums negative by the microscopic smear test, made this report appear highly advisable even though the original intention of the experiments did not include such plans.

We have now completed studies (to be published shortly) with nutrient mediums for small plantings of tubercle bacilli and have compared these with the sterile potato cylinder originally recommended. It has been found that simple inspissated glycerol (3 per cent) water (25 per cent) egg yolk medium without the addition of broth or other diluents compares favorably with the potato medium. It was for this reason that the potato medium and egg yolk medium were used in these studies. In order to further check the mechanical manipulations, preparation of media, and performance of tests, all specimens were tested by two independent and separate groups of trained workers who also used separate incubators. One hundred suspected sputums negative by microscopic examination were used for the tests. These were obtained from cases in which routine monthly smear examinations had been made. Of these 32 per cent had never been positive by smear during residence in the sanatorium, 5 per cent had not been positive for over two years, 11 per cent had not been positive for over one year, and 52 per cent had been negative from four to twelve months prior to culture test.

The specimens tested by culture were carefully examined in smear to determine that they were negative for the purpose before proceeding to use them for this experiment. The method used for destroying contaminants consisted briefly of mixing 1 c.c. of a well homogenized sputum (homogenized by means of an electric vibrator beater) with 15 c.c. of 5 per cent pure oxalic acid solution. After shaking well, the mixture was incubated (at  $37^{\circ}C$ ) for about forty-five minutes (previous tests having shown that a time leeway within sixty minutes is permissible). One sample was then washed with ten volumes of sterile 0.9 per cent sodium chloride solution and centrifuged while another was neutralized to  $P_H$  7.0, using bromthymol blue as indicator and centrifuged. The sediment, after decanting of the supernatant liquid, was again mixed with 0.9 per cent saline solution and planted on a number of tubes of potato and egg yolk medium. Sodium hydroxide as a reagent to destroy contaminants, was tested in various ways with the same specimens of sputum. It was used in equal volume and 15 volumes, of 3 per cent and was left unneutralized (only washed), and in comparable tests was neutralized with sulphuric acid solution.

The pertinent results of this study are included in Tables I and II.

An examination of Table I reveals that neutralization is not necessary when dealing with sputum. However, tests with tissues and blood so alter the conditions

TABLE I

A COMPARISON OF THE WELKALY POSITIVE RESULTS OBTAINED BY TWO SEPARATE GROUPS OF WORKERS WITH ONE HUNDRED MICROSCOPIC NEGATIVE SPUTUMS USING OXALIC ACID AND POTATO OR EGG MEDIUM AS TEST METHOD

METHOD USED	NEW POSITIVE FINDINGS OBTAINED AT WEEKLY READINGS						TOTAL POSITIVES
	2 wk	3 wk	4 wk	6 wk	9 wk	12 wk	
Oxalic acid— potato medium	1*	5	25	9	7	3	50
	0	9	25	12	4	1	51
Oxalic acid— neutral with NaHCO <sub>3</sub> — potato medium	1	11	14	14	9	1	50
	0	8	33	8	4	1	54
Oxalic acid— egg yolk medium	4	18	13	8	3	1	47
	2	21	7	11	2	3	46
Oxalic acid— neutral with NaHCO <sub>3</sub> — egg yolk medium	3	23	13	3	9	6	57
	1	23	14	7	2	1	48

\*The upper figure throughout the table records the positive findings by one group of workers while the lower figure records those of the second group

that with these materials, neutralization is necessary. The data on this will be published shortly. The character and amount of residue from acid treated sputums make early reaction adjustment from the neutral nutrient medium possible, which is not the case with tissues and blood.

It is also to be noted that the two groups of workers obtained approximately the same percentage results in spite of the lack of uniformity of emulsion specimens due to the nature of the tuberculous material excreted in the sputum. It is also to

TABLE II

THE SIGNIFICANCE OF CONTAMINATIONS IN TESTING ONE HUNDRED NEGATIVE MICROSCOPIC SPUTUMS FOR TUBERCLE BACILLI

METHOD USED	NUMBER OF TUBES CONTAMINATED*	COMPLETELY CONTAMINATED OF A TOTAL OF 100 SPUTUMS	TUBES POSITIVE FOR TUBERCLE BACILLI*
Oxalic acid— potato medium	14**	0	125
	8	0	136
Oxalic acid— neutral with NaHCO <sub>3</sub> — potato medium	12	1	128
	12	1	135
Oxalic acid— egg yolk medium	24	3	109
	24	0	119
Oxalic acid— neutral with NaHCO <sub>3</sub> — egg yolk medium	30	2	118
	14	1	129

\*Each group used 300 tubes of culture medium for the 100 sputums and for each method. The total tubes of culture medium used by both groups for all the tests were 2400.

\*\*The upper figure given in the tabulations represents the findings of one group of workers while the lower figure represents the findings of the second group of workers.

be noted that the potato cylinder medium and the inspissated egg yolk medium both proved to be good nutrients and about equal in value, by quantitative tests with fine suspensions of tubercle bacilli, yielded about the same percentage positive findings with the negative microscopic sputums

Since the elimination of contaminations is an important phase of the procedure of isolating pure cultures of tubercle bacilli for diagnostic purposes the data presented in Table II are particularly pertinent in revealing the value of oxalic acid solution for this purpose

The agreement noted between the two groups of workers both in contaminations and positive findings for tubercle bacilli was quite striking in view of the empiric nature of the material tested. It figured in percentage of the total tubes contaminated it was for the first group 7.2 per cent, and for the other 4.8 per cent, while the positive cultures for tubercle bacilli obtained by the first group was 39.9 per cent of the tubes of medium used, and by the second group was 43.2 per cent. It is interesting also that only six sputums of the 100 sputums examined by four methods by the first group contaminated totally by one of the methods, and that for the second group only two sputums contaminated by one of the methods. In no case was the individual sputum contaminated in all four methods.

The empiric nature of the tested material, the sputums, is attested by the results in contaminations as well as in positive cultures obtained, bearing out the well known physical nature of tuberculous material. This is further borne out by the fact that although the percentage positive findings by each group was about equal, this equality attained as a result of the fact that five sputums found negative by the first group were positive for the second, and another five sputums were positive for the first group and negative for the second. In many cases (15 per cent of the sputums) where twelve tubes were planted using four methods, only one tube revealed a positive culture. If the positive results of the two groups of workers using the four methods were taken in total 71 per cent of the 100 sputums were positive by culture.

In the case of apparently negative findings we have occasionally found that a careful scrutiny of the surface of the media would yield a positive solitary macroscopic colony, which easily passes unobserved in poor light or with hasty superficial examination. This was particularly so for the potato medium but also was true with the egg yolk medium. Trained workers rarely failed to detect such colonies. In verification of earlier observations<sup>2</sup> the tests with 3 per cent sodium hydroxide to destroy contaminants proved this reagent to be far less efficient than the oxalic acid. Because of the contaminations, in addition to the greater toxicity of the sodium hydroxide for the tubercle bacilli, the percentage positive findings for tubercle bacilli were considerably less than the tests with oxalic acid. The contaminations in this series of sputums treated with sodium hydroxide neutralized with sulphuric acid ran from 16 per cent on the potato medium to as high as 52 per cent on the egg yolk medium. For this reason a tabulation of the results with the sodium hydroxide method was omitted.

#### DISCUSSION AND CONCLUSIONS

In practical clinical medicine a positive diagnostic finding is in the majority of cases of far greater significance than a negative observation. It was previously

pointed out that the finding of tubercle bacilli in pathologic materials might be considered in the light of a certified diagnosis in tuberculosis<sup>1</sup> provided the error of calling all acid fast bacilli, tubercle bacilli be guarded against. Specimens from certain regions of the body yielding acid fast bacilli such as is exemplified in sputum usually prove reliable in being designated as tubercle bacilli. Therefore, the microscopic findings in stained smears from certain regions assume practical importance when positive. The fact, however, that it requires the presence of large numbers of bacilli (at least 100 000 per c c) in a specimen before they can be readily discerned in the stained smear makes negative findings by this method obviously extremely unsatisfactory from a clinical or public health standpoint, and makes the so called negative specimen assume importance. The highly susceptible guinea pig and delicate culture method both have proved valuable for discerning tubercle bacilli as contrasted to acid fast bacilli and especially for discerning the presence of small numbers (as few as 10 to 100). The culture method possesses the advantage of ready accessibility and economy of performance, but both the guinea pig and culture method have the disadvantage of requiring time for obtaining results. Yet the time element in a chronic disease does not assume the proportions it would in an acute disease and cannot counteract the importance of reliable positive findings. The importance of applying the more sensitive culture test as compared to the microscopic smear test is evident when it is realized that 71 per cent, in this series, of sputums which had been negative by smear for intervals of from four months to over two years proved positive on a single examination by culture. There is no doubt that a repetition on another specimen from the 29 cases negative on single test would probably reveal additional of these as positive, in view of the fundamental nature of tuberculous material.

#### SUMMARY

Of 100 sputums, negative, for from four months to over two years on monthly reexamination by the microscopic smear test, 50 per cent were found positive by a single culture test. When several delicate culture methods (potato medium and egg yolk medium) were used and the studies were carried out by two groups of workers, 71 per cent positives were obtained. The finding of positives by one group and not by another is understandable on the basis of the lack of uniformity of empiric specimens, due to the nature of the tuberculous material. This makes accurate scientific observations with routine sputa of little value for comparison of methods or media unless verified by accurate quantitative studies. It does not, however, vitiate the practical importance of the positive findings in a high percentage of clinical specimens found repeatedly negative by smear, and suggests a more extended use of culture methods in diagnostic and public health work in spite of the apparent disadvantage of time delay. The importance of repeating a test, when negative, is obvious and requires no comment.

The technical details of this study were conducted with the assistance of C. D. Vidal, L. D. Miller, Ray Stoner and Martha Brown.

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## UNBREAKABLE TUBES FOR SEROLOGIC WORK AND FOR THE SHIPMENT OF BLOOD SPECIMENS\*

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TO ELIMINATE breakage in transit and in the laboratory, I have devised a light-weight, inexpensive, and unbreakable tube for the shipment of blood specimens

The tube is made of nonexplosive, though combustible celluloid. It measures 75 by 15 mm and holds 10 c.c. of the blood. A properly softened cork stopper, quality XX, size 5, short, fits into this tube firmly enough to prevent any leakage in

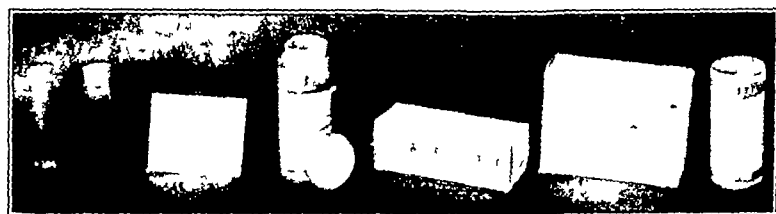


Fig 1—Unbreakable tube and mailing carton

transit regardless of rough handling. I have tested the dependability of the tube and the security of the cork stopper by throwing the loaded mailing container vigorously against the ceiling of the laboratory and allowing it to drop to the floor, and by throwing it from one end of the room to the other with force. The tube was found unharmed, and the stopper securely in place. No leakage was observed.

The blood can be kept in the washed and sterilized tubes from one to three days without bringing about any change in the original immunologic properties of the specimens. Tubes 75 by 10 mm made of the same material were used in carrying out complement fixation (cold overnight incubation) tests, and tubes 75 by 7 mm were used in the precipitation tests. The results could be read with the same ease as in the glass tubes. No hemolytic or anticomplementary reactions were experienced in the case of complement fixation tests and no pseudo-precipitations were observed.

The tubes are cheaper than the glass tubes, if purchased in large quantities, and since they are not fragile they last indefinitely, and afford a worth-while economy.

When first obtained, the tubes are soaked in warm soapy water, gently swab-

\*From the Clinical Laboratory, Public Health Institute  
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bed with a soft hair brush and thoroughly rinsed inside and out in a continuous stream of tap water. The tubes are then placed mouthside down into galvanized wire baskets and drained to dryness. The baskets are then placed inside a specially constructed sterilizing chamber. On the bottom of this sterilizer is a shallow enamel tray, into which is placed a folded cotton towel or sheeting. This is soaked with full strength formaldehyde, and the door of the sterilizer is tightly closed. The tubes are left under the influence of the vaporized formaldehyde for twelve to twenty-four hours. This period was found to be ample for the complete sterilization of the tubes. A sufficient number of cork stoppers, thoroughly washed, contained in a cotton gauze sack are sterilized at the same time. At the end of twelve to twenty-four hours the tubes and the stoppers are removed from the sterilizing chamber and the tubes are carefully stoppered.

Each tube is equipped with an identification blank. It is properly packed in a container lined with a piece of corrugated paper. This is placed in a carton, and is ready for shipment. Used tubes are cleaned and sterilized as described above.

It must be remembered that these tubes, though not explosive, are combustible. They must not be dried inside or over the dry-air gas oven, nor must they be exposed to the open flame. The tubes can be sterilized as many times as may be required by the formaldehyde-vapor method, but will not stand repeated sterilization by steam or by boiling.

Fig 1 shows the complete outfit necessary for mailing blood specimens.

## A METHOD OF COLLECTING AND PRESERVING SMALL BLOOD SAMPLES FOR GLUCOSE DETERMINATIONS\*

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THE present study was undertaken in connection with the glucose tolerance tests of insurance companies. A method was desired, the accuracy of which would not depend on a definite measurement of the sample at the time of collection. In the procedure offered the technic for collecting is simple and may be undertaken by an untrained person. For this reason it may have applications other than its present one. A diabetic patient, for example, should find it as easy to take a fingertip sample of his blood as to give himself an insulin injection. To diabetic specialists it is suggested as an easy way to keep in touch with patients living at a distance. It may also be found a serviceable device to have in a hospital laboratory for collecting and transferring samples.

A 0.1 cc capillary pipette lined with preservative salts is used for the collection, the blood being sealed inside the pipette by means of a rubber band stretched over the ends. For this purpose the usual Folin micro-blood pipette<sup>1</sup> is shortened by cutting off the blunt end, and an extra graduation is added about 3 cm beyond the 0.1 cc mark. The blood sample is collected to this second graduation and the measurement is left to the analyst at the time of testing. The procedure is as follows: Make the lancet puncture in the finger-tip, allow the blood to flow into the

\*From the Biochemical Laboratory of the Metropolitan Life Insurance Company.  
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pipette, tip it back and forth a few times to dissolve the preservative lining and attach the rubber band. At the time of making the analysis measure the sample by discarding the excess blood and analyze it in the same way as a fresh sample.

The pipettes selected were 16 cm. long and it was found that the corresponding rubber bands must be 1 cm. in width and 9.5 cm. in length. If longer or narrower the bands did not fit the pipettes snugly and permitted some evaporation of the blood column inside. If shorter they were too tight and might be punctured by the tips of the pipettes.

As a preservative Lax and Szimari<sup>4</sup> recommended the use of sodium fluoride and mercuric chloride. According to their report these two salts, in the proportion of sodium fluoride, 1 per cent, and mercuric chloride, 0.1 per cent, prevented glycolysis in blood samples for periods up to a month and had no reducing effect on the testing reagents.

Our investigation was complicated somewhat by the difficulty of getting a measured amount of the salts into the pipette. Aqueous solutions were found impracticable because the drying process was so slow at room temperature, and if oven drying was resorted to the solution expanded and a large proportion overflowed from the openings of the pipette. For this reason it was decided to try a suspension instead of a solution and the sodium fluoride was precipitated from a concentrated aqueous solution by the addition of 95 per cent alcohol. The suspension could then be sucked into the pipette to the second graduation, since this was to be the measure of blood collected, and with the aid of a towel applied at the tip most of the alcohol-water mixture could be drawn off, leaving the deposit of salt inside. This could then be quickly distributed and dried by passing a gentle current of air along the bore of the pipette.

The mercuric chloride, however, being soluble in alcohol, could not be so precipitated. When the two salts were dissolved together in water, and alcohol was added, some of the mercuric chloride was brought down, but in a reduced state, and on standing further reduction with consequent darkening of the precipitate was noted. Blood samples treated with this precipitate showed a loss in glucose value indicating that the mercuric chloride had not been available.

It was then decided to adopt the procedure outlined above for the fluoride salt but to add the mercury in alcohol solution to the previously precipitated fluoride suspension. And because most of this alcohol solution would be poured out of the pipette after its introduction, the concentration of the mercury was increased from 0.1 per cent to 1 per cent. Enough then remained in the pipette after decanting to approximate the desired amount.

#### PREPARATION OF THE SUSPENSION SOLUTION

Dissolve 0.5 gm. of sodium fluoride in 15 c.c. of water and rinse into a 50 c.c. volumetric flask, using as little water as possible for the rinsing. Add 95 per cent alcohol until the flask is about half full and the sodium fluoride is brought down as a fine precipitate.

Dissolve 0.5 gm. of mercuric chloride in 3 c.c. of 95 per cent alcohol and add this to the fluoride suspension, making up the volume with additional alcohol.

The preservative thus prepared will show only a slight deposit of mercury and will be suitable for use for at least a month if kept in a dark cupboard.



## PREPARATION OF THE PIPETTES

Shake the suspension thoroughly then pour 2 or 3 cc into a small beaker. Agitate the beaker to make sure that the salt is uniformly dispersed and draw the liquid and suspension into the pipette to the second graduation. As many pipettes as desired may be filled at one time, the suspension in the beaker being stirred well with each pipette before it is filled and the pipettes laid horizontally after filling. Each pipette in turn is then tipped slightly toward the blunt end so that the suspension particles are drawn away from the tip then tilted slowly toward the tip so that the liquid begins to flow away from the salt. When the liquid reaches the opening at the tip of the pipette apply a cloth or filter paper to draw it off, being careful not to absorb any of the salt. If the precipitate runs toward the tip too swiftly tilt once more toward the other end before continuing to decant at the tip. It is easier to see the precipitate and to regulate its progress if the pipette is held in front of some black surface as a background. When the salt is concentrated into a space a little less than a cm in length at the tip of the pipette, direct a gentle current of air through the tip from a rubber tube. This will cause the wet salt to flow back along the bore of the pipette. Care must be taken to see that none is blown out the opposite end. Continue the gentle blowing until the salt is dried in a thin, even lining for the pipette. It requires only about a minute's time to line a pipette in this way, and once prepared and dried it may be kept indefinitely before using.

## COLLECTION OF THE BLOOD

Make the lancet puncture in the finger-tip and collect the blood in the usual way for the micro method, allowing it to flow into the pipette to the second graduation and being careful not to admit bubbles. Then tip the blood column from one end to the other in the pipette, three times in each direction, in order to dissolve the preservative. Finally let the blood run down to the tip again, insert the tip of the pipette into a fold of the rubber band and stretch the band over the opposite end, pressing it down snugly over the blunt end. If the edges of the blunt end are not well covered some evaporation of the blood column toward that end may occur.

The bands should be washed with water and wiped dry before using.

If the first drop of blood fails to enter the pipette readily, wipe the tip with a cloth moistened in alcohol.

## MAKING THE DETERMINATION ON PRESERVED BLOOD

Remove the rubber band and with a dampened cloth wipe off any blood on the outside of the pipette. Then with a slender wire such as the one inside a syringe needle, loosen the blood inside the tip. With the tip end held up, shake the pipette downward until the blood column begins to fall, then reverse the pipette and allow the blood to flow toward the tip again. Repeat this process until the blood cells, which may have settled along one side or at one end, are again mixed with the plasma. Then run the blood down to the tip once more, lower the meniscus to the 0.1 cc mark and blow the measured blood into the diluting fluid. Rinse the pipette repeatedly. Finish the sugar determination according to the Folin method.<sup>1 2 3\*</sup>

\*These methods have been summarized in a recent paper by Folin New England J Med 296 727 1932. The sodium sulphate recommended in the precipitating reagent has not been used in our determinations since it was impossible to prevent hemolysis of the preserved blood samples.

The ease with which the blood begins to flow depends to some extent on the length of time since it was collected and also on the weather. The blood will remain more loose and fluid in the pipette at lower temperatures than during the very warm months.

In some cases it will be found that small patches of blood will adhere to the inside of the pipette and the rinsing is then very important. It will be found either to

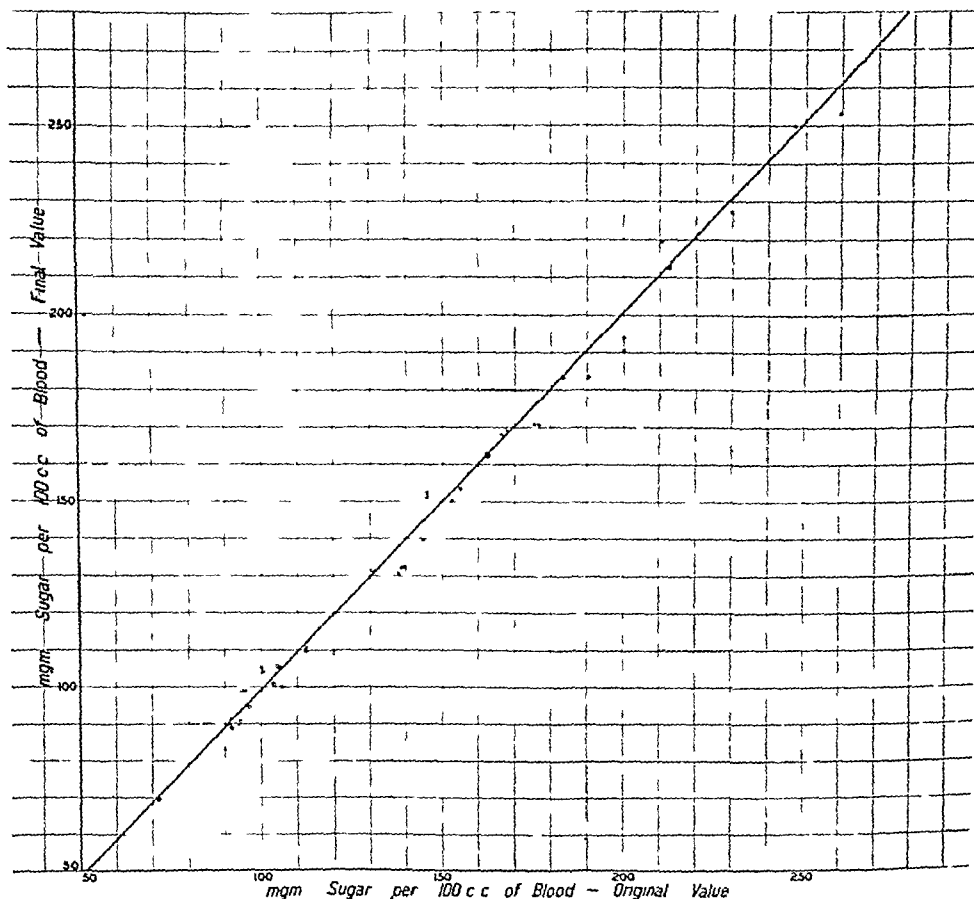


Fig 1 — The scatter diagram shows the results of the second test of each sample plotted in rectangular coordinates against those of the first. It is apparent that the scatter points fall rather closely around a line at forty-five degrees of the coordinate axis. The fact that the angle at which the line is inclined is forty-five degrees means that the first and second tests give on an average the same results, and the fact that the points cluster closely around the line is the expression of the fact that this relation is rather closely followed throughout the series of tests. The line has been laid by the method of least squares. (Alfred J. Lotka.)

dislodge the blood or to extract its glucose. A stiff piece of rubber tubing, 4 or 5 cm in length, may be conveniently attached to the pipette to serve as a mouth piece for the rinsing.

#### EXPERIMENTAL RESULTS AND DISCUSSION

A series of blood specimens were kept from one to fourteen days and showed sometimes a slight drop, as often a slight increase over the corresponding fresh samples. The practice was to collect two samples of the same blood, one to be ana-

lyzed at once, the other to be kept in the pipette. Out of 88 comparisons made during the spring and summer of 1931, only 11 showed an error of more than 5 per cent. The greatest error encountered was 7.5 per cent. The figures for these comparisons are given in Table I. A graph has been prepared through the courtesy of Dr. Alfred J. Lotka to represent the facts brought out by the experimental data (Fig. 1).

TABLE I

A COMPARISON OF THE GLUCOSE VALUES OF FRESH SAMPLES AND PRESERVED SAMPLES OF THE SAME BLOODS

NO	DAYS KEPT	GLUCOSE IN MILLI GRAMS PER CENT		PER CENT OF DEVIATION	NO	DAYS KEPT	GLUCOSE IN MILLI GRAMS PER CENT		PER CENT OF DEVIATION
		FRESH SAMPLE	PRESERVED SAMPLE				FRESH SAMPLE	PRESERVED SAMPLE	
1	5	217.5*	213.9*	1.7	45	10	136.9	141.8	3.6
2	5	100.8*	97.1*	3.7	46	8	96.6	94.8	1.9
3	7	84.9*	86.8*	2.2	47	8	202.0	212.7	5.2
4	1	90.1*	90.9*	0.8	48	8	103.8	100.0	5.5
5	6	189.5*	192.4*	1.5	49	10	100.0	105.2	5.2
6	6	229.9	227.3*	1.1	50	7	137.9	130.7	5.2
7	6	94.6*	99.0	4.7	51	6	101.0	102.0	1.0
8	6	152.6	150.4	1.4	52	6	210.5	219.7	4.4
9	6	132.5*	137.9	4.1	53	5	163.9	166.6	1.6
10	6	98.0	101.5	3.5	54	11	94.3	90.9	3.6
11	6	206.1	202.0*	2.0	55	10	138.8	132.4	4.6
12	6	135.1*	132.9*	1.6	56	10	169.4	167.3	1.2
13	6	91.9*	93.9*	2.2	57	9	99.5	101.5	2.0
14	4	176.9	182.9	3.4	58	9	200.0	206.1	3.1
15	4	92.0*	95.1*	3.4	59	9	175.4	166.6	5.0
16	3	183.4	183.4*	0.0	60	9	162.5	162.5	0.0
17	6	168.0	169.4	0.8	61	9	104.7	105.2	0.5
18	6	96.6	97.6	1.0	62	6	204.0	215.0	4.9
19	6	142.7	150.3	5.3	63	10	119.7	121.8	1.8
20	6	90.1	83.3	7.5	64	10	113.6	115.6	1.8
21	6	120.4	124.2	3.2	65	6	112.3	110.5	1.6
22	6	178.5	175.4	1.7	66	6	90.5	90.1	0.4
23	6	200.0	190.4	4.8	67	6	156.2	149.2	4.5
24	6	133.3	129.0	3.2	68	6	190.4	183.4	3.7
25	4	241.0	232.5	3.5	69	8	103.1	101.0	2.0
26	6	181.8	176.9	2.7	70	12	104.1	105.8	1.6
27	6	145.9	141.8	2.8	71	11	192.4	198.0	2.9
28	12	95.7	99.0	3.5	72	7	139.8	132.4	5.3
29	6	90.9	86.2	5.2	73	5	91.3	96.1	5.3
30	6	145.9	151.4	3.8	74	6	155.0	153.7	0.8
31	6	130.7	131.5	0.6	75	6	91.3	89.7	1.8
32	6	114.9	116.9	1.7	76	6	91.3	91.3	0.0
33	1 hr	71.6	69.7	2.7	77	6	145.9	152.6	4.6
34	6	95.2	97.6	2.5	78	5	99.5	94.8	4.7
35	10	124.9	131.5	5.3	79	10	181.8	186.9	2.8
36	8	105.2	105.2	0.0	80	6	175.4	168.0	4.2
37	8	212.7	212.7	0.0	81	6	266.7	274.0	2.7
38	8	247.0	250.0	2.0	82	6	303.0	317.5	4.7
39	8	175.4	170.9	2.6	83	6	259.7	253.1	2.5
40	10	119.0	114.9	3.4	84	10	112.3	109.8	2.2
41	9	149.2	147.0	1.5	85	7	166.6	168.0	0.8
42	11	93.0	87.3	6.1	86	9	144.9	139.8	3.5
43	8	100.5	104.1	3.6	87	7	103.6	100.5	3.0
44	8	178.5	186.9	4.7	88	4	162.5	162.5	0.0

\*This figure is an average of duplicate determinations.

The keeping period is apparently limited only by the beginning of evaporation of the blood column. It is easy to tell by inspection if evaporation has occurred to any extent. If so the value will tend to be high. It has seldom been noted under eight days even in warm weather and showed only very slightly after fourteen days in the winter.

The procedure was adopted by this Company for tolerance tests in October, 1931 and has been used since then with acceptable results in all cases where the collection was made according to instructions. A grooved wooden box holding the pipettes, bands and a lancet is placed inside a pasteboard carton for mailing.\* Six of these boxes, containing three samples each, were sent to San Francisco to be returned as a test of their travelling qualities. Four of the boxes showed the samples in as perfect condition on return as when mailed. In two of the boxes a slight separation in the blood column toward the blunt end of the pipette occurred. This

TABLE II

THE GLUCOSE VALUES OF BLOOD SAMPLES SENT THROUGH THE MAIL AND OF THE CORRESPONDING FRESH SAMPLES COLLECTED AT THE SAME TIME

BOX NO	DATE SENT	DATE ANALYZED	GLUCOSE MILLIGRAMS PER CENT	
			MAILED SAMPLE	FRESH SAMPLE
1	Feb 11	Feb 19	92.6	97.6
			183.4	180.1
			66.8	68.5
2	Feb 15	Feb 29	129.0	129.8
			124.9	
			129.0	
3	Feb 17	Feb 29	90.9	94.8
			212.7	215.0
			92.6	100.0
4	Feb 17	Feb 29	155.0	157.4
			156.2	
			162.5	
5	Feb 17	Feb 29	88.9	88.9
			176.9	180.1
			50.3	56.3
6	Feb 24	Mar 4	91.7	90.9
			169.4	161.2
			107.0	110.5

did not interfere with the analysis, since the separated blood could be tipped out before the sample was measured. Results of the analyses of these samples are given in Table II.

Since the method has given acceptable results for glucose preservation, plans are now being made to adjust the procedure to the determination of urea.

\*The outfits now in use were made especially for the purpose by the Empire Laboratory Supply Company, New York, and satisfactory rubber bands were obtained from the Hodgman Rubber Company, Malden, Mass.

## SUMMARY

A practical method has been devised for collecting small samples of blood and preserving their glucose content. The samples are collected and kept in capillary pipettes sealed by means of rubber bands. As a preservative the pipettes contain a small amount of sodium fluoride and mercuric chloride, introduced in the form of a suspension-solution. Blood samples may be kept from one to fourteen days or until evaporation begins. In a large majority of cases the glucose value will remain constant within an error of 5 per cent.

The author is indebted for helpful advice to Dr. N. R. Blatherwick who suggested this problem and who helped to adapt the method to the purposes of the Company.

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## A METHOD OF PREPARING BLOOD SMEARS\*

KATSUJI KATO, M.D., CHICAGO, ILL.

NOTHING is more essential than the proper preparation of the smears in the routine examination of the blood, as well as in a study of the morphology of the blood cells. As even the best trained technicians in large hospitals pay little attention to the proper method of preparation, many blood smears are so poorly made that they prove to be wholly inadequate for even the ordinary differential count, not to speak of the more exacting cytologic study. The average clinicians who are frequently compelled to make the smears themselves are even more careless in this important procedure. I feel that this is not so much a matter of experience, as it is a matter of the use of a proper method.

In the literature on hematologic technic, I find that only the two most commonly employed methods are described.<sup>1</sup> They are (1) Ehrlich's two-cover-glass method, and (2) the two slide method. Both of these time-honored methods are unsatisfactory. The first of these gives a fairly good result in the hands of experienced persons, but it has many disadvantages. The cover glasses are thin and small, causing much difficulty in manipulation and often loss of the specimen due to breakage. The two-slide method is utterly defective in its fundamental principle, as recently brought out by Silberstern.<sup>2</sup> By this method the distribution of the leucocytes is greatly distorted or they are even pushed away to the edges of the slide. The painstaking work of Gyllensward<sup>3</sup> only emphasizes the unsatisfactoriness of the old methods.

The method to be described here was shown me by Dr. William Bloom in the Department of Anatomy of the University of Chicago, who developed this technic. Dr. Bloom tells me that he is not aware of its being used by anyone else, but that he is unwilling to claim the method as his own, for the procedure is so simple that it must have occurred to other workers with the dry smear technic.

\*From the Department of Pediatrics, the University of Chicago.  
Received for publication May 19, 1932.

Although I have never seen this technic used by others, I think it desirable to report this method because it is a very convenient means for obtaining a uniform distribution of the cellular elements of the blood.

A clean slide is used to receive a drop of blood flowing from a fresh stab wound. The drop should not be too large, the optimum size being from 3 to 5 mm in diameter when received on the slide. The initial position of the drop on the slide is of some importance. It should be slightly to one side away from the center of the slide. An oblong cover glass, 21 by 50 mm is laid immediately over the drop-

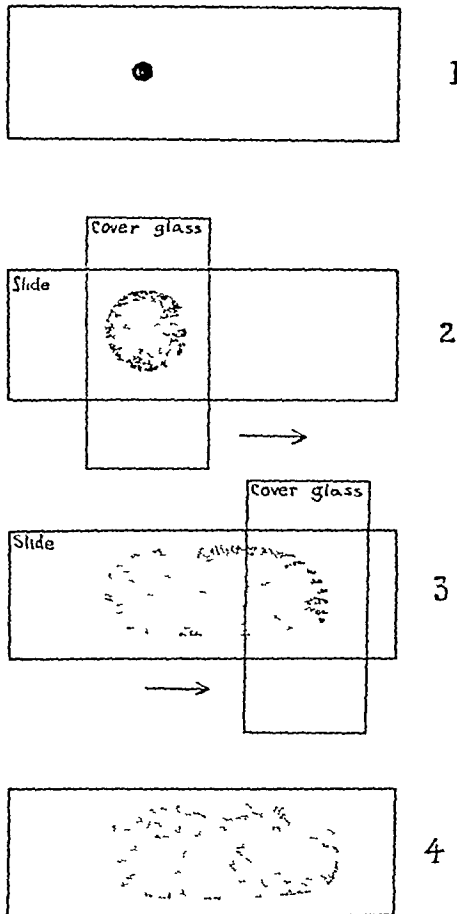


Fig 1—Showing steps of preparing blood smear

let and the blood is allowed to spread between the cover glass and the slide into a circle of thin film by the weight of the cover glass. No pressure should be exerted over the cover glass for this purpose. When the blood has spread into a circle of appropriate dimension, the cover glass is quickly drawn across toward the other end of the slide, as illustrated in Fig 1. This will give an elongated oval of thin film, the border of which should be well within the edges of the slide. The slide should be fanned at once and air dried in the usual manner. The film so prepared will be found to have an even distribution of the leucocytes in almost every microscopic field. The erythrocytes will also be found to be spread in a single layer without overlapping or rouleau formation.

The thickness of the film may be easily controlled, after a little experience, by the length of time which is allowed to elapse from the moment the cover glass is applied until it is pulled across. As a rule, the longer the time, the thinner the film, and vice versa. The blood of a leucemic patient will have to be spread rather thinly, as the leucocytes are usually numerous, while leucopenic blood should be spread thickly. Viscosity of the blood will also affect the thickness of the film. It is difficult, for example, to make a thin film from the blood of a newborn infant due to its greater viscosity. It will be found that if the cover glass is allowed to remain too long, it will adhere to the slide, causing difficulty in further manipulation. The plethoric or hydropic blood often found in leucemias and certain other blood dyscrasias needs to be worked with moderate speed, or the film will tend to become too thin. The blood of certain mammals, the reptiles and the amphibians, containing very large corpuscles, must be spread rather thick, commensurate with the greater diameter of the blood cells. Each worker will have to find out the finer details and particulars from personal experience.

This method is made public for two reasons. (1) Because the literature on hematology commonly consulted by both clinicians and technicians fails to mention this method, and (2) because it gives uniformly good blood smears.

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## AN AIR MOTOR FOR STIRRING\*

ARLINGTON C KRAUSE, PH D, M D, BALTIMORE, MD

THE following air motor was devised as a substitute for the electric motor. Fluctuation of the direct current of the clinical laboratory caused a racing of the electrical stirring apparatus, which resulted in the destruction of glass beakers and other vessels. In use, the air motor has demonstrated many advantages over the electric motor. It has been found to be light in weight, quickly ad-

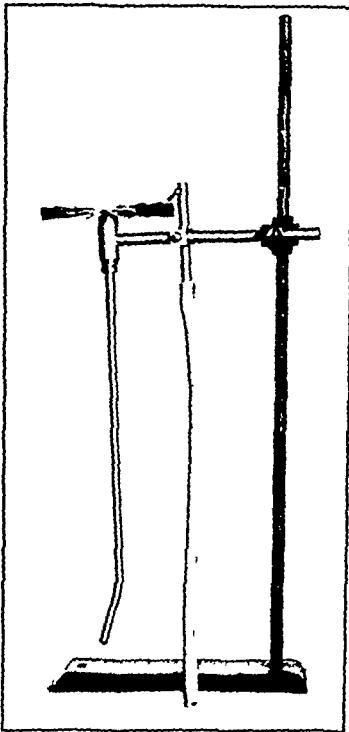


Fig 1

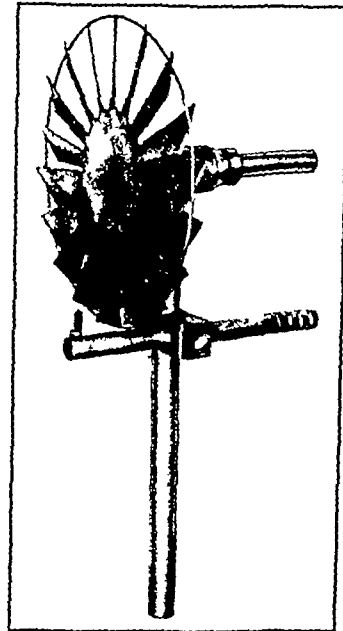


Fig 2

justable to any angle, cheaply made, and vibrationless. It takes a small amount of air and may be run constantly day and night with little or no care.

### APPARATUS

A 4-inch fan was made from a circular piece of No 24 gauge brass plate, cut radially  $1\frac{1}{4}$  inches to make 18 blades. The blades were turned  $30^\circ$ , and fastened to a circular brass wire for stability. The fan was soldered onto a fixed brass collar, which was attached to a brass shaft 3 inches long and  $\frac{1}{4}$  inch in diameter.

\*From the Wilmer Ophthalmological Institute of the Johns Hopkins Hospital and University.  
Received for publication May 21, 1932.



Directly below the collar, the shaft was passed through the vertical part of a T shaped brass holder, which was used as a journal. A hole was bored into the side of the journal for the purpose of oiling. The horizontal part of the T-shaped holder acted as a side arm. Another collar, attached to the shaft by a setscrew, was placed below the holder to keep the shaft in place. The lower end of the shaft was made into a female screw with a beveled shoulder to hold stirring rods and other attachments. Holes were drilled transversely through the shaft and through the stirring rods to aid in disconnecting them. The motor described above was operated by means of compressed air which was passed through a rubber hose into a vertical brass tube, which was attached by a setscrew to the side arm of the holder. The air was then directed over the fan through a 1/16 in. air nozzle, which was set into the side of the tube at a 45° angle. This air motor was supported by clamping the side arm to a laboratory stand.

The stirring rods were made of brass and monel metal with male screw ends. A glass stirring rod was constructed by cementing a glass rod into the hollow tip of a short metal rod having a male screw end. A flask, attached to a straight stirring rod by means of a rubber stopper and then rotated by the motor, was also used for agitation. Large flasks were easily turned in a water bath because of the buoyancy of the water.

The air motor described above was an improved design of another model which was easily constructed. The fan was constructed from the top of a discarded ether can. Blades of the fan were made by cutting the top peripherally in radial sections 5 cm deep and 1 cm wide. The blades were turned 45° by means of pliers. A one holed rubber stopper holding a glass stirring rod was placed in the central opening of the fan. A journal for the stirring rod was made from a one-holed rubber stopper, which held a piece of glass tubing. The journal was supported by a laboratory clamp. Compressed air from a glass nozzle revolved the fan. This simple type of air motor was useful for many operations for which an electric motor was too expensive.

## THE CALIBRATION OF MICROPIPETTES BY A COLORIMETRIC METHOD\*

FRANKLIN C BING, PH D , CLEVELAND, OHIO

THE increased use of micro methods of blood analysis necessitates a fairly rapid and convenient technique for testing the accuracy of the small pipettes used in such work. Calibration by the classical method, weighing the amount of mercury delivered, is often a tedious process and may be attended with several sources of error that are obviated only by taking unusual care. During the past two years we have used a simple colorimetric procedure for the calibration of pipettes having capacities varying between 0.0005 cc and 1.000 cc. This method possesses the theoretical advantage that the calibration is performed with whole blood, the fluid which most of the pipettes are intended to measure. With the gravimetric procedure, it is frequently necessary to allow a correction for the difference in volume between a meniscus formed by mercury and that formed by water or blood. The colorimetric method automatically eliminates such calculations.

### METHOD

Several cubic centimeters of fresh oxalated or defibrinated blood are obtained. From the well mixed specimen a sample is removed with a pipette calibrated by standard methods to contain 1 cc. The blood is allowed to run into a clean and empty 2 liter flask, the tip of the pipette being held against the glass. The blood in the neck of the flask is washed down with a few cubic centimeters of distilled water. The pipette is rinsed into a beaker, and the washings quantitatively transferred to the flask. In adding water to the flask it should be allowed to run down the sides to avoid the formation of foam. Dilution is made to the mark and the contents are thoroughly mixed. Using calibrated apparatus, this process yields a standard blood solution of accurately known dilution.

The pipette to be calibrated is carefully cleaned, dried, and filled to the mark with the same blood from which the standard solution is prepared. The pipette should be calibrated in precisely the same way as it is to be used, either *to deliver* or *to contain*, the latter method being preferred. The blood is diluted with sufficient water to make the final volume about 2000 times the nominal capacity of the pipette. Thus, 0.1 cc of blood is diluted to 200 cc, using a calibrated volumetric flask, and 0.001 cc of blood is transferred into 2 cc of water, measured with an accurate pipette into a clean, dry test tube. With volumes smaller than 0.001 cc, the approximate dilution factor of 2000 is neglected, the final volume being maintained at 2 cc because 1 cc is required for the colorimetric determination. There is thus obtained a blood solution of which, in all cases, the final volume is known but the exact dilution is unknown.

\*From the Department of Biochemistry, School of Medicine, Western Reserve University.  
Received for publication May 24, 1932.

Hemoglobin estimations are made upon both samples of diluted blood by the benzidine method <sup>1, 2</sup> The colors produced are compared in a colorimeter

### CALCULATIONS

The actual capacity of the pipette is obtained by substituting the proper numerical values in the following equation

$$C = \frac{S \times v_1 \times b}{R \times v_2}, \text{ in which}$$

C = the capacity of the pipette

S = the colorimeter reading of the blood used as standard

R = the colorimeter reading of the blood of unknown dilution

$v_1$  = the total volume of diluted blood of unknown dilution

$v_2$  = the total volume of diluted blood used as standard

b = the actual volume of whole blood employed in preparing the standard dilute blood

In practice, the values for S and R are best determined by triplicate or quadruplicate determinations To illustrate by an actual example a pipette supposed to hold 0.020 c.c. is calibrated, the final volume of the diluted blood being 50 c.c., and the standard consisting of 1.005 c.c. of blood diluted to 2000 c.c. The observed colorimeter readings of three standard tubes, the first being set at 10 mm., are 10, 10, and 10 mm. Three tubes prepared from the blood of unknown dilution give readings of 12.2, 12.2, and 12.3, average 12.2 mm. The capacity of the pipette is calculated as follows

$$C = \frac{10.0 \text{ mm} \times 50.00 \text{ c.c.} \times 1.005 \text{ c.c.}}{12.2 \text{ mm} \times 2000 \text{ c.c.}} \\ = 0.0206 \text{ c.c.}$$

### ACCURACY OF THE METHOD

In Table I are presented data comparing the capacities of several pipettes as determined by the present method and by means of the usual procedure, weighing mercury In each case, the figures in the table represent either averages obtained

TABLE I  
COMPARISON OF CALIBRATIONS WITH COLORIMETRIC METHOD AND WITH MERCURY

PIPETTE NO	COLORIMETRIC METHOD C C	GRAVIMETRIC METHOD C C
1	1.000	1.005
2	0.1104	0.1096
3	0.1022	0.1011
4	0.0262	0.0262
5	0.00664	0.00662
6	0.00571	0.00572

from colorimetric readings in triplicate or quadruplicate on a single dilution of blood, or the average of two weighings of mercury It is apparent that the differences in the values obtained by the two methods are not more than 1 per cent, which is entirely satisfactory for many practical purposes

An independent check on the accuracy of the calibration is afforded by com-

parison of the colorimetric hemoglobin estimation of a second blood sample with the value obtained by the oxygen capacity method of Van Slyke and Neill.<sup>5</sup>

Duplicate calibrations by the colorimetric method have yielded values that differ from each other by not more than 1 per cent. With pipettes having capacities less than 0.001 c.c., however, it is difficult to attain this order of accuracy unless the size of the capillary is so chosen that the length is sufficient to minimize the error of reading the meniscus. It is obvious that the accuracy of the method renders it of no special value, except, perhaps, that of convenience, for the calibration of pipettes having capacities greater than 0.1 c.c. It has, however, been satisfactorily employed in standardizing micropipettes intended for the determination of hemoglobin, sugar, and other constituents in blood.

#### APPLICATION TO THE CALIBRATION OF DILUTION PIPETTES USED IN THE ENUMERATION OF BLOOD CELLS

The value of the present method is revealed by the ease with which the dilution pipettes used in hemacytometry can be calibrated. Even though the bulb of such pipettes may have a volume considerably greater than 0.1 c.c., calibration by the colorimetric method has proved more reliable than the gravimetric method in our hands, due to the difficulty encountered in trying to fill the pipette. This is partly due to the fact that the glass bead present in the ampulla floats on the mercury.

These pipettes can also be calibrated by weighing the amount of water which they remove from a partly filled and counterpoised weighing bottle. The U. S. Bureau of Standards tests the calibration of dilution pipettes by a volumetric method with special apparatus.<sup>4</sup> It may be noted that the allowable error of the dilution ratios, according to the present specifications of the Bureau,<sup>3</sup> is, for white cell pipettes,  $\pm 3.5$  per cent, and for red cell pipettes,  $\pm 5$  per cent. In other words, if the erythrocytes are enumerated in a blood specimen having a true count of 500 million per cubic millimeter, the value obtained with certified pipettes may be anywhere between 475 million and 525 million, inclusive, omitting all errors except those of the measuring pipettes. While such variations would never lead to erroneous clinical interpretations, yet, for accurate experimental work, we have found it necessary to calibrate all red cell dilution pipettes at the 0.5, 1.0, and 101 marks. For this purpose the present method has proved entirely satisfactory.

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## THE COMPARATIVE EFFICIENCY OF THREE STAINS FOR TUBERCLE BACILLI\*

C J KOERTH, M D, AND R J B HIBBARD, M D, SAN ANTONIO, TEXAS

IT IS a well recognized fact among laboratory workers that various acid fast stains vary in efficiency for the routine examination of sputum for tubercle bacilli. Also various modifications of the same stain vary in results. Brown and Hayes<sup>1</sup> found that the use of brilliant green instead of methylene blue gave an increase of 8 per cent in positive findings.

Furthermore, it is recognized that at various stages of growth the tubercle bacillus varies in its acid fast quality, depending on the media and length of time it is grown on the same media.<sup>2</sup>

There are nonacid fast, weakly acid fast, and strongly staining acid fast bacilli.

In this Hospital it has been the custom to use these stains Ziehl-Neelsen, Schulte-Tigges,<sup>3</sup> and Spengler.<sup>4</sup> These stains are all used on the same specimen of sputum.

The technique as we do them are as follows:

### 1 Ziehl-Neelsen

Stain with carbol fuchsin five minutes

Wash with water

Decolorize in acid alcohol eight minutes

Wash with water

Counter stain thirty seconds with 1 per cent methylene blue

### 2 Schulte-Tigges

Stain with carbol fuchsin one minute

Wash with water

Decolorize with 10 per cent aqueous solution of sodium sulphite (until color practically disappears)

Wash with water

Counter stain with saturated aqueous solution of picric acid

Wash with water, dry, and examine

By this method the bacilli are red against a yellow background

### 3 Spengler

Stain with carbol fuchsin (warm but not hot enough to bubble) five minutes

Pour off stain and without washing

Pour on picric acid alcohol four to five seconds (1 part saturated solution aqueous picric acid 1 part 95 per cent alcohol)

Wash with 60 per cent alcohol

Treat with 15 per cent nitric acid till yellow (not over thirty seconds)

Counter stain with picric acid alcohol solution until lemon yellow

Wash with distilled water, dry, and examine

Bacilli are brownish red against a yellow background

These statistics comprise the results of several years' work, and were formed through curiosity and without prejudice in favor of any stain.

\*From the Woodmen of the World Hospital  
Received for publication May 27, 1932

Each slide was examined in ten random fields except where such fields were negative, in which case a thorough search of the entire slide was instituted

As a majority of our cases are far advanced the numbers of bacilli are more abundant than would be found in institutions limiting patients to first and second stage cases

There are, however, enough cases in which bacilli are absent or few to evaluate the tests in cases with but few bacilli. We also feel the series is sufficiently large to compensate for any errors due to the human element, variations in thickness of smear, etc

TABLE OF EXAMINATIONS

Cases examined	600
Slides examined 3 by 600	1,800
Fields examined 10 by 1,800	18,000
Total Number of Bacilli Counted	
1 Ziehl Neelsen	19,692
2 Schulte Tigges	51,759
3 Spengler	71,559
Total	143,010

Grossly, it will be seen that the Schulte-Tigges is almost three times as efficient as the Ziehl-Neelsen, and the Spengler almost four times as efficient. This does not, however, prove the latter two stains are worth the extra trouble of using them, as the vast majority of slides showed abundant numbers of bacilli with any of the three stains

Therefore, a comparison of the negative slides is in order

1 Ziehl Neelsen	166
2 Schulte Tigges	144
3 Spengler	140

It would appear then that by using Spengler's method one would get twenty-six positives for every one hundred sixty-six negative slides by the Ziehl-Neelsen method—an advantage of 12 per cent

The advantage of Spengler's method in facilitating search for the bacilli is further shown by the statistics of negative fields in slides in which bacilli were somewhere found

	Ziehl Neelsen	Schulte Tigges	Spengler
Fields negative in positive slides	427	137	104
To which should be added 10 fields for totally negative slides	1660	1440	1440
Total	2087	1577	1504

Thus, out of 2087 negative Ziehl-Neelsen fields Spengler's method showed 2087-1504 or 583 positive fields, an advantage of over 25 per cent

As can readily be seen the Schulte-Tigges is but slightly less efficient than the Spengler. However, it is a much more difficult stain to perform and the chance of error in technique is large. The sodium sulphite must be absolutely fresh, and must not be used for more than a very few slides before being changed

It will be argued that the Ziehl-Neelsen is the better stain for the routine examination of sputum by nonlaboratory men. Undoubtedly it and its modifica-

tions have stood the test of time against many newer methods. Certainly it is more difficult to over decolorize with acid alcohol than with nitric acid. However, the use of nitric acid is for so short a time (thirty seconds) that it can readily be timed by the watch accurately and without in any way being onerous. Whereas, the acid-alcohol method requires considerable waiting. We feel that students with a little practice could do this stain well and save time.

Following the technic in use here it takes approximately sixteen minutes to do a Ziehl-Neelsen stain and seven minutes to do a Spengler. The saving of time is considerable. Where a Ziehl-Neelsen technic using acid and then alcohol is employed the time consumed would be approximately the same in either case.

#### CONCLUSIONS

1 It is evident that in old chronic third stage cases expectorating large numbers of bacilli that a far greater percentage of bacilli have to a certain extent lost some of their acid fastness (and probably some are completely nonacid fast), as compared with cases of but slight involvement expectorating smaller numbers of bacilli. The comparison of the total Ziehl-Neelsen (19,693 bacilli) with the total Spengler (71,559 bacilli) adequately illustrates this point.

2 That Spengler is a better stain from standpoint of actual results in showing the presence of tubercle bacilli, is adequately shown by these statistics.

3 The Ziehl-Neelsen technic has no advantage in respect to the time saving element, and if acid-alcohol is used is actually much more tedious.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A KILDUFFE, M D, ABSTRACT EDITOR

## HYDATID MOLE A New Method of Diagnosis and Prognosis, Mayes, B M J Australia II 20 603, 1932

The author calls attention to the fact that in the presence of a hydatid mole the urine contains enormous quantities of anterior pituitary hormone (200,000 to 500,000 mouse units per liter)

The following procedure is based upon this fact

A morning specimen of urine is diluted with distilled water 1 50 in a test tube and 1 100 in another test tube

An Aschheim Zondek test is done with each dilution and also with the undiluted urine

If the reaction is positive with the undiluted urine alone the case is one of pregnancy, but if positive with either of the above dilutions, the reaction is interpreted as positive for hydatid mole

## POLIOMYELITIS Resistance to, Jungeblut, C W, and Engle, E T J A M A 99 2091, 1932

1 The widely accepted hypothesis which postulates that the resistance of adults to poliomyelitis is due exclusively to universal subclinical immunization against the virus is inconsistent with a number of epidemiologic observations bearing on the age distribution in various latitudes and the recent trend towards an advance in the age line

2 The assumption of a unique immunizing power of the virus in the absence of disease production cannot be verified experimentally

3 The opinion that natural virucidal substances in the serums from human adults can develop only through previous contact with the specific antigen is open to criticism because of their apparent formation in man and monkeys under conditions which preclude the presence of the virus

4 The evidence in support of the current concept that the incidence of virucidal serums in human adults stands in direct relation to the density of population should be reviewed since individual serums may show wide discrepancies in virucidal power, which seems to vary with the blood group and to fluctuate with changes in the physiologic state of the body

5 In a restricted number of instances it has been possible to demonstrate that virus neutralizing substances may occur in the serum of immature monkeys after prolonged treatment with anterior pituitary extracts or similar principles recovered from the urine of pregnant women

6 The suggestion is offered that the mass protection enjoyed by the adult human population rests primarily on the normal function of the endocrine balance characteristic of mature age

## Urinary Antisepsis, Davis, E, and Sharpe, J C J A M A 99 2097, 1932

As determined by antiseptic tests made before and after administration, pyridium, given in capsules to normal persons in unit maximum dosage (0.4 gm), colors the urine  
Caprokol (dose, 0.75 gm) judged by the same standard, exerts an irregular and transient antiseptic action in about one third of the four hour samples

Methenamine (dose, 1 gm) is incomparably more efficient than either pyridium or caprokol in causing the normal person to secrete urine which is antiseptic against both the colon bacillus and the staphylococcus



Acridavine (dose 0.2 gm) administered in capsules, exerts an antiseptic action in normal urine against both the colon bacillus and the staphylococcus, which is uniform and consistent to a surprising degree. Urinary alkalinity is essential.

Acridavine (dose, 0.2 gm) administered in shellac covered pills, is practically inert.

Acridavine, administered in capsules, although noninjurious in efficient dosage, causes unpleasant symptoms (nausea and catharsis) in a fair proportion of cases.

Acridavine, for the reason given, has its definite clinical limitations. Clinical experience indicates, however, that this drug is of distinct value in selected cases, particularly those of acute infections of the urinary tract.

**LEPROSY** The Demonstration of in the Blood by Means of the Thick Drop Method, Sardjito, M., and Sitanala, J. B. Med Dienst d Volksgez in Nederl Indie 21 33, 1932.

The authors confirm the demonstration of lepra bacilli in the blood by the thick drop method as used for malaria, using, of course, the Ziehl-Neelsen stain.

**B C G** A Study of the Pathogenicity of, Feldman, W. H. Am J Path 8 755, 1932.

Using a strain of B C G obtained from Calmette or the Pasteur Institute, a deliberate attempt was made to increase its pathogenicity by subculturing the organism on a glycerinated egg medium. Transfers were made every thirty days. From each succeeding subculture 4 guinea pigs were given injections, 2 intracerebrally, 1 subcutaneously, and 1 intraperitoneally. The report deals with data obtained after the organism had been subcultured on glycerinated egg medium for fifteen generations.

Or a total of 58 guinea pigs inoculated, lesions histologically indistinguishable from those of genuine tuberculosis occurred in the tissues of 11, and cultures of acid fast bacilli were obtained from each. Although the majority of the lesions occurred in animals that had been given intracerebral injections, 1 animal that was given an intraperitoneal injection and another given a subcutaneous injection died with lesions of a tuberculous nature. So far, attempts have failed to promote a succession of tuberculous lesions by the reinoculation into guinea pigs of infective material from lesions.

The particular strain B C G studied is not devoid of pathogenicity for guinea pigs, and the assertion that the organism is innocuous cannot be accepted without reservations.

Subculturing the organism on glycerinated egg medium at monthly intervals for a period of fifteen generations did not markedly enhance its virulence.

**TISSUE STAIN** Silver Impregnation of Glia and Nerve Fibres in Paraffin Sections After Formalin Fixation, Wilder, H. C. Am J Path 8 785, 1932.

The following method has been found satisfactory.

**Fixation and Embedding** Fix tissues in 10 per cent formalin, wash in tap water, dehydrate in alcohol, clear in chloroform, and embed in paraffin.

**Bromuration** Pass paraffin sections through xylol and graded alcohols, rinse in distilled water, and place in 34 per cent hydrobromic acid for thirty minutes.

**Sensitization** Wash in distilled water ten to twenty seconds and flood the slide with 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

**Impregnation** Wash in distilled water ten to twenty seconds and place for twenty seconds in silver diamino hydroxide.

To 5 cc of 10.2 per cent silver nitrate add ammonium hydroxide drop by drop until the precipitate which forms is dissolved. Add 5 cc of 3.1 per cent sodium hydroxide and just dissolve the resulting precipitate with a few drops of ammonium hydroxide. Make the solution up to 50 cc with distilled water.

**Reduction** Wash in distilled water two seconds and agitate each slide separately in the following reducing solution until it ceases to give off a brown cloud.

Distilled water 50 cc, 40 per cent neutral formalin (neutralized with magnesium carbonate) 0.5 cc, 1 per cent uranium nitrate 1.5 cc.

**Counterstaining and Mounting** Wash in distilled water counterstain with eosin, dehydrate in alcohol, clear in xylol and mount in Canada balsam. Argentation frequently allows hematoxylin to be used as a nuclear stain, but bluing must take place in tap water, as ammonia dissolves the silver.

Distilled water is used in the preparation of all solutions. The uranium nitrate solution and the 10.2 per cent silver nitrate keep indefinitely and the silver diamino hydroxide keeps for a week or more in amber, glass stoppered bottles. The impregnating and reducing solutions retain their activity in Coplin jars for two days. The hydrobromic acid may be kept in a Coplin jar and used repeatedly for an indefinite time. It is important that the ammonium hydroxide be kept in a well stoppered bottle.

**ALLERGY, Value of Cytologic Examination of the Nasal Smear in Differential Diagnosis of Infection and, Kahn, I. S., and Stout, B. F. J. A. M. A. 99: 1494, 1932**

The following conclusions are presented:

1. A positive eosinophilic nasal smear of from 10 to 90 per cent is almost invariably diagnostic of allergic rhinitis.
2. A positive eosinophilic smear of 4 per cent or over is highly suggestive of allergic rhinitis.
3. Conditions such as a pure, thin, watery discharge, allergic quiescence and, at times, intercurrent infections in previously definitely proved cases of allergic rhinitis are proof that the absence of eosinophilia at a single examination does not bar allergic rhinitis.
4. The presence of abundant non-eosinophilic polymorphonuclear leucocytes in the smear is practically diagnostic of infection.
5. Allergic and infectious rhinitis and sinusitis may exist coincidently or at alternate periods in the same person.
6. This simple nasal smear test, if done as a routine measure, will prove of decided diagnostic value to rhinologists and to workers on allergy.

**LEUKEMIA, Some Immunologic Aspects of, Hulper, W. C., and Russell, M. Arch. Int. Med. 49: 113, 1932**

This study was undertaken to test the theory that the increase of leucocytes in leucemias might be referable to a disturbance in the antiproliferative activity of the blood serum. The following conclusions are advanced:

1. The antiproliferative quality of leucemic plasma is not decreased, but slightly increased, as compared with that of normal human plasma. Myeloid leukemia is therefore not the result of the decrease or absence of this quality of the serum.
2. Myeloid leucemic plasma inhibits the emigration of the granulocytic elements from the explanted clot of blood leucocytes.
3. Antileucocytic serum produced by the intravenous injection of leucemic leucocytes into rabbits impairs the emigrative and proliferative qualities of normal and leucemic leucocytes in tissue cultures.
4. Tissue cultures of leucocytes allow a rather accurate titration of the cytotoxic qualities of an antileucocytic plasma.
5. Isolated favorable therapeutic results (in 4 of 11 cases) with nontitrated antileucocytic serum in leucemias seem to indicate that properly prepared and titrated antileucocytic serums may offer an effective remissive method for the treatment in leucemias.

**CANCER, Interrelations Between Histologic Structure and Blood Chemical Findings in, Hulper, W. C., Woodward, G. E., and Fry, E. G. Am. J. Cancer 15: 2666, 1932**

1. The malignancy indices and the blood  $P_H$  values of 34 cases of carcinoma were correlated and a preponderance of histologically highly malignant tumors was found in patients with an alkalosis, while the tumors of histologically low malignancy predominated in patients with a normal blood  $P_H$ .
2. The alkalosis of the blood in cancer patients varies not only with the extent of the disease, but also with the degree of malignancy and maturity of the tumor.
3. The degree of cellular irregularity of cancerous tissue is increased in patients with an alkaline  $P_H$  of the blood as compared with patients with a blood  $P_H$  in the normal range.
4. In about one third of the cancer patients with a hyperglycemia (which was present in 78.3 per cent of our cases) a high number of mitoses was found, while this phenomenon was absent in cancers with a normal blood sugar.

**AUTOHEMAGGLUTINATION, Hyperproteinemia as a Cause of,** Reimann, H. A. J A M A 99 1411, 1932

In a case of myeloma without Bence Jones proteinuria, the clue to the underlying condition was obtained by an inability to count the erythrocytes because of immediate rouleau formation. As a result of this phenomenon, difficulties were encountered in preparing blood smears and in selecting a suitable donor for blood transfusion. Similar phenomena are encountered in other conditions associated with hyperproteinemia, as in pneumonia, kala azar and pregnancy. Marked hyperproteinemia was present in the case of myeloma here reported. It is suggested that, in this case, disease of the bone marrow may be largely responsible for the increase of globulin and fibrinogen.

**WATER. Study of Bacteriological Methods of Testing and Means of Disinfecting Water With Chlorine With Particular Reference to Swimming Pool Water,** Mallmann, W. L., and Carey, W. Am J Pub Health 23 35, 1933

The authors report that samples of swimming pool water collected during periods of use and tested immediately showed more pollution than duplicate samples handled in the usual manner by storing and testing later.

During periods of use, swimming pool water showed marked pollution as measured by colon bacilli and streptococci indices in the presence of residual chlorine contents of 0.2 to 0.5 p.p.m.

During periods of rest, the pollution evidenced during use, disappeared. The rate of disappearance depended upon the type of treatment used.

With chloramine treatment, a delayed germicidal action occurred. This was also true of chlorine treatment in alkaline water, but to a lesser extent.

The colon bacilli and streptococci indices roughly parallel each other. The preponderance of the colon bacilli and streptococci incidences varies.

A sodium thiosulphate treated sample bottle is recommended for collecting pool samples.

The method advised for collection of samples follows:

The bottle is prepared by adding a small crystal of sodium thiosulphate to a moist bottle or 0.5 c.c. of tenth normal sodium thiosulphate to a dry bottle subsequent to sterilization by moist heat. Care must be taken not to rinse the bottle in collecting the sample. The sodium thiosulphate added is sufficient to neutralize a residual chlorine content of at least 1 p.p.m. The results obtained from sodium thiosulphate treated bottles and from poolside tested samples are similar.

**TYPHUS FEVER, Laboratory Diagnosis of Endemic and Rocky Mountain Spotted Fever,** Badger, L. F. Am J Pub Health 23 19, 1933

There are certain and definite criteria for the identification of an unknown strain of virus as being one of endemic typhus or spotted fever.

With the exception of the involvement of the external genitalia of the male guinea pig the clinical symptoms produced in laboratory animals by the virus of endemic typhus exhibit nothing characteristic of the disease. In the male guinea pig the western virulent virus of spotted fever produces a characteristic involvement of the external genitalia, while the viruses of the eastern spotted fever studied produce no symptoms in the guinea pig which are not produced by some known bacteria. Both the western virulent virus and the eastern virus of spotted fever may produce in the rabbit the characteristic involvement of the external genitalia and in the monkey the characteristic exanthem.

The viruses of spotted fever, as well as the virus of endemic typhus, produce in the brain of the guinea pig the nodal and vascular lesions seen in European louse borne typhus.

The Weil-Felix reaction has been found positive with approximately 75 per cent of the sera of rabbits and monkeys inoculated with either the endemic typhus virus or the western virulent spotted fever virus. The Weil-Felix reaction has been found positive with but approximately 25 per cent of the sera of monkeys inoculated with the eastern spotted fever virus.

Immunity tests must be clean cut and made in both directions.

Cultures of the cardiac blood of animals from which transfers are made must be con-

sistently negative, as confusing clinical symptoms and immunity tests have been caused by known bacteria

**PREGNANCY, New Hormonal Reaction in, Hofmann, H** *Zentralbl f Gynäk* 56 2534, 1932

The author advocates the method following

Withdraw 25 c.c. of blood by venipuncture, separate the serum, shake it with ether. The ovaries of a not less than a 2,300 gm rabbit are inspected by a laparotomy and 13 c.c. of serum injected intravenously

The ovaries are again inspected in twenty four hours for the presence of hemorrhagic spots which indicate a positive reaction

**TUBERCLE BACILLI, Concentration of in Sputum, Nassi, E** *Policlinico* 39 1649, 1932

Reagent Magnesium hydrochloride 2 gm, sodium bicarbonate 6 gm, distilled water 1000 c.c.

Method Mix equal parts of sputum and reagent and stir slowly with a glass rod for 10 minutes. Transfer to a large test tube and, while slowly stirring, heat gradually in a water bath at 85 to 90° C.

Pour in a sedimentation cup (or centrifuge). After three hours (if sedimented), pour off the supernatant liquid and make smears from the sediment. Stain as usual.

A second rapid method is thus described

Prepare a 15 per cent solution of gelatin and filter while hot

Heat the sputum fifteen minutes in a water bath and when it is a homogeneous mass, to 10 c.c. of melted gelatin add 5 c.c. of heated sputum and 5 c.c. of ligroin in a test tube. Cork the tube and shake thoroughly for thirty seconds.

Place the tube in cold water until the gelatin solidifies. Then pour off the ligroin, scrape the surface of the gelatin and use the scrapings for smears.

The author recommends 4 per cent sulphuric acid for decolorization and well diluted methylene blue for counterstaining.

**TUBERCULOSIS, Costa and Red Cell Sedimentation in Laryngeal, Rubinstein, C** *Am Rev Tub* 27 92, 1933

An evaluation of the Costa reaction and a comparison with the red cell sedimentation reaction are presented, the material used for this purpose being 75 cases of pulmonary tuberculosis complicated by laryngeal tuberculosis.

Before instituting any radical form of treatment it is highly important that the phthisic laryngologist secure accurate data regarding the patient's pulmonary process and his immunobiological status.

The author recommends as a determinant of pulmonary activity, in addition to sedimentation reaction, the Costa reaction.

The Costa and sedimentation reactions disclosed a complete parallelism in cases of far advanced disease. In latent cases and those with moderately advanced disease there was a disparity between the two reactions that favored the Costa in sensitivity.

Both reactions were found to be of value in differential diagnosis between simple and tuberculous laryngitis.

Tuberculosis of the larynx widens the indications for chest surgery which, when applied at the proper immunobiological phase, is a most powerful weapon in effecting a complete cure of laryngeal tuberculosis.

It is the author's opinion that the Costa reaction may be employed profitably in the laryngeal tuberculosis clinic as an objective method for decision as to activity and as a guide in the treatment to be employed.

**DEXTROSE, Normal Renal Threshold for, Campbell, R. A., Osgood, E. E., and Haskins, H. D** *Arch Int Med* 50 952, 1932

The authors studied 34 persons, 22 of whom gave satisfactory results. Their conclusions were that filtrates obtained by Somogyi's zinc sulphate sodium hydroxide method of precipitating blood proteins give true sugar values within a range of 7 mg per hundred cubic centimeters.

Filtrates obtained by the tungstate precipitation method give results within a narrow range, averaging 21 mg higher than the true sugar, hence, tungstate methods are clinically satisfactory, and this factor, 21 mg, can be used to transpose results from one method to the other.

It seems to be impossible to produce an alimentary glycosuria in some normal persons by the ingestion of large amounts of dextrose.

The renal threshold for true sugar varies from 99 to 228 mg per hundred cubic centimeters, 80 per cent of cases having values that range from 140 to 190 mg.

If a normal renal threshold is to be given, it must allow for wide variation. The authors suggest, from the probable error of 15.5 mg and the mean of 151 mg of this series, a probable range of from 105 to 200 mg for true sugar, or from 125 to 220 mg if the tungstate precipitation method is used.

#### **BLOOD, Macroscopic Examination of, Wintrobe, M. M. Am. J. M. Sc. 185: 38, 1937**

The following procedures are advocated and described.

By a series of consecutive steps which are simple and may be quickly performed, it is possible by the use of a single instrument, a modified hematocrit, to gain accurate and valuable information concerning the blood.

1 Five cubic centimeters of blood are drawn from a vein with a dry syringe and needle and placed in a small bottle containing 10 mg of potassium oxalate with which the blood is thoroughly mixed.

2 The hematocrit is filled to the "10" mark by means of a capillary pipette and sedimentation rate is determined. The sedimentation rate serves as a guide to the presence and intensity of organic destructive or infectious disease.

3 The filled hematocrit is next centrifugalized until no further packing of the corpuscles takes place. The volume of packed red corpuscles, and the volume of packed leucocytes and platelets may then be read.

The volume of packed red cells is an accurate index of the degree of anemia and is more easily and more accurately measured than even of the red cell count. It also serves to correct the sedimentation rate of the blood, as above determined, for the influence of anemia or polycythemia. Furthermore, if the red cell count and hemoglobin value of the sample of blood collected for hematocrit studies has been determined, the mean volume and hemoglobin content of the red corpuscles may be calculated. The latter information is of great value not only in differentiating the anemias but also in serving as a guide to the type of treatment which may be expected to be effective.

The volume of packed white cells and platelets serves as a rough guide to the quantity of these corpuscles in the blood.

4 Finally, the icterus index of the plasma may be read and this, in the absence of liver disease, biliary obstruction and carotinemia, serves as a useful guide to the degree of blood destruction.

#### **DEXTROSE TOLERANCE, Decreased in Acute Infectious Diseases, Williams, J. L., and Dick, G. F. Arch. Int. Med. 50: 801, 1932**

The series studied consisted of one hundred and eight patients, including sixty-seven patients with scarlet fever, seventeen with diphtheria, eight with pneumonia, five with influenza, three with acute tonsillitis, three with measles and one each with erysipelas, encephalitis, mumps, epidemic meningitis and poliomyelitis. Ten normal subjects are also included.

The authors state that glycosuria occurred in 41 per cent of patients with acute infectious diseases. The largest average amount of dextrose was excreted by the patients with influenza and miscellaneous acute infections.

This glycosuria is accompanied by a lower carbohydrate tolerance, as shown by dextrose tolerance tests and blood sugar curves both in acute infectious diseases and in experimental infections in animals.

Administration of insulin improves the dextrose tolerance in acute infections.

This work suggests that in infectious diseases there is often an injury to the islets of Langerhans with a lessened production of insulin.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughan, Professional Building, Richmond, Va

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### Streptococci in Relation to Man in Health and Disease

THE student of modern bacteriology, indeed, even the casual observer of the variety and multiplicity of its recent advances must perforce feel that many of its later developments have primarily emphasized the inherent vastness and complexity of the subject.

Particularly may this be said to be true in the study of that exceedingly extensive and important group, the streptococci, the importance of which is related to the production of disease and the complexity of which as concerns their species specific activities and differentiation is only recently becoming fully appreciated.

Intrited, perhaps by the outstanding contributions of Brown in the establishment of the basic classification now generally accepted, the literature upon the streptococci has assumed mammoth proportions culminating in the exceedingly comprehensive work of the Thomsons working in the Pickett Thomson Laboratories.

To correlate all this work and to present it in a succinct yet comprehensive fashion is the purpose of Dr. Williams' book.

That some such attempt has been greatly needed will readily be granted. That it has been done by one so well fitted by experience and extensive personal investigation in this field assures the reader of a well planned and equally well executed volume practically indispensable to the library of the bacteriologist as well, indeed, to all interested in the study of disease.

The first three chapters present a general resume of the subject and include a historical survey and classification, a discussion of general characteristics and their utility in the differentiation of species, and the incidence and general effect of these organisms on man.

Subsequent chapters are devoted to the relation of streptococci to local infections, to general infections, to streptococci in erysipelas, in scarlet fever, in septic sore throat, to a discussion and tentative classification of Beta hemolytic types, to streptococci in rheumatism, arthritis, and allied conditions, in diseases classed as virus diseases, and, finally, to general considerations.

The bibliography of twenty seven pages is appended.

The format and general make up of the book are in keeping with the productions of this publisher. Typographical errors are exceptionally few.

There can be no doubt that this volume is a contribution of the first importance, that it will be received with acclaim by bacteriologists in general, and that it will remain as a standard reference for some time to come.

It can be recommended without reserve.

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### The Sputum—Its Examination and Clinical Significance†

IT IS quite true, as the author states, that with exception of certain routine studies such as search for tubercle bacilli, examination of the sputum is becoming to a certain extent a lost art, with a consequent failure to obtain information that is of paramount diagnostic significance in certain cases.

The author in this monographic volume has compiled and correlated various observations on examination of the sputum so that they are readily available in compact form. It is interesting however after careful perusal of the volume to note that little of new has been added to our knowledge of the sputum in the recent years.

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\*Streptococci in Relation to Man in Health and Disease. By A. W. Williams M.D. First Assistant Director Bureau of Laboratories Department of Health New York. Cloth 260 pages 7 plates. Williams & Wilkins Co. Baltimore Md.

†The Sputum—Its Examination and Clinical Significance. By Randall Clifford M.D. Associate in Medicine Peter Bent Brigham Hospital Assistant in Medicine Harvard Medical School Formerly Associate Physician and Director of Pulmonary Clinic Massachusetts General Hospital. Cloth 167 pages. The Macmillan Company New York 1932.

The author's microscopic routine consists in the preparation of four slides, one unstained, another stained for tubercle bacilli, the third with Smith's Gram Loam Stain and the fourth with the Fontana Stain for Vincent's organisms. He very properly emphasizes the importance of search for parasitic fungi.

### Clinical Atlas of Blood Diseases

THE book, arranged as a convenient small handbook, contains a series of most excellent colored plates representing the blood smear in various diseases. The first eight plates depict the various blood elements, erythrocytic and leucocytic, normal and abnormal, which may appear in the circulating blood, arranged in the order of their developmental sequence. Plate 1 depicts the development of granular leucocytes, Plate 2 of lymphocytes, Plate 3 of the erythrocytes. Plate 4 depicts the varieties of myeloblasts and promyelocytes, Plate 5 of the granular leucocytes, Plate 6 megakaryocytes and normoblasts, Plate 7 other abnormal cellular elements of the blood, and Plate 8 types of neutrophilic granular leucocytes. The remaining thirty plates depict typical pictures in the various blood diseases.

For reference value both in teaching and in diagnostic work this compact volume will find a very useful place.

### Anatomy and Physiology

BUNDY'S Textbook of Anatomy and Physiology for Nurses, edited by Dr. Weeder, is now in its sixth edition. Its arrangement follows very much that of preceding editions and the volume can be well recommended for the use of nurses and others who wish to acquire a good, general knowledge of the structure of the human body and its functions. Anatomy and physiology are correlated throughout the book rather than being kept separate and distinct.

### Biochemistry in Internal Medicine†

A COMPACT volume on normal and abnormal physiology as it can be studied clinically by biochemical methods. Of value as a students' textbook and a reference manual. Subject matter includes carbohydrate, protein and cholesterol metabolism, the metabolism of chloride, calcium and phosphate, acid base balance, respiratory exchange, diabetes, renal function, hepatic function, pancreatic function and kindred subjects. The last chapter contains a concise outline of clinical chemical changes in various disorders, indicating the nature of studies that should be made in each of the diseases and the findings usually observed.

### Man and Microbes‡

A MOST enjoyable written primer for the laity on the history of bacteriology and its application in agriculture, industry, public health, and medicine. Abundantly illustrated and written by an authority on the subject. Not too long.

\*Clinical Atlas of Blood Diseases. By A. Piney, M.D., M.R.C.P., Director of Pathological Department, The Cancer Hospital, London. Consulting Pathologist, Chelmsford Hospital and Stanley Wyard, M.D., M.F.C.P., Physician, The Cancer Hospital, London, and Princess Beatrice Hospital. Second Edition. With 38 illustrations, 34 in color. Cloth, pages 105. P. Blakiston's Son and Company, Inc., Philadelphia, 1932.

\*\*Textbook of Anatomy and Physiology for Training Schools and other educational institutions, by Elizabeth R. Bundy, M.D., Formerly Adjunct to the Professor of Anatomy in the Woman's Medical College of Pennsylvania and Superintendent of Connecticut Training School for Nurses, New Haven, etc. Sixth Edition, Revised and Enlarged by S. Dana P. Weeder, M.D., Instructor in Anatomy, University of Pennsylvania. Cloth, 446 pages. P. Blakiston's Son and Company, Inc., Philadelphia, 1930.

†Biochemistry in Internal Medicine. By Max Trummer, Ph.D., Clinical Chemist and Toxicologist, formerly in charge of the Laboratories of Biochemistry of the Jefferson Medical College and Hospital and of the Psycho-Biochemistry Laboratory, Graduate School of the University of Pennsylvania, and Abraham Cantarow, M.D., Instructor in Medicine, Jefferson Medical College, Assistant Attending Physician, Philadelphia General Hospital, in charge of Laboratory of Biochemistry, Jefferson Hospital. With a foreword by Elmer H. Funk, M.D., Sutherland M. Prevost, Professor of Therapeutics at Jefferson Medical College. Cloth, pages 454, with illustrations. W. B. Saunders Company, Philadelphia and London, 1932.

‡Man and Microbes. By Stanhope Bayne Jones, M.D., Professor of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. Cloth, pages 128, illustrated. The Williams and Wilkins Company and Associates in Cooperation with The Century of Progress Exposition, Baltimore, 1932.

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## EDITORIAL

### Suprarenal Hemorrhage in the Newborn

**A**LTHOUGH at any age the adrenal glands are essential to life, there is evidence that they subserve a special function during the antenatal period. In the fetus the adrenal, like the liver, is proportionately very much larger than after birth. At birth it is about one-third the size of the kidney, while in the adult it is about one-thirtieth of the size of the kidney. While the individual growing to adult size increases in weight about twenty times, the adrenal glands increase only to about double their original weight. The antenatal adrenal is especially rich in cortical tissue, the secretion of which as we shall see appears to serve an especially important rôle prior to birth.

Immediately after birth pronounced structural changes become manifest in the organ. The inner or central cortical layers disintegrate and are replaced with vascularized connective tissue. Only a very small portion of the cortex immediately beneath the capsule escapes this degenerative process. The medulla appears to grow into the new connective tissue framework, replacing the former inner layer of the original cortex.



These changes are clearly recognizable in the first two weeks of life. They are slow however and do not reach their greatest stage until about the end of the first year. Regeneration of cortical material commences in the second year and according to Goldzieher and Gordon does not reach full development until adolescence.

These authors in a review of the subject conclude that in all probability the cortical hyperplasia in fetal life is connected with the respiratory metabolic needs of the fetus. They believe that large amounts of cortical material are necessary during fetal life to maintain tissue respiration. After birth, with the onset of respiration and the increase of oxygen tension in the blood, lesser amounts of cortical hormone are required to maintain tissue respiration and the cortex therefore involutes to some extent. The oxygen tension in the blood is higher after birth than prior thereto. Comparable observations have been made in cyanid poisoning and beriberi. In both conditions tissue respiration is interfered with and there results a compensatory hypertrophy of the adrenal cortex. Apparently the adrenals attempt to produce more cortical hormone, to overcome the impaired tissue respiration in chronic cyanid poisoning.

The suprarenal gland is one of the most vascular tissues if not the most vascular in the body. Each minute six times its weight of blood passes through the organ. It is not surprising therefore that occasionally during the involutionary process of this extremely vascular organ hemorrhages occur which may become sufficiently extensive to be productive of symptoms. Massive suprarenal hemorrhage in the newborn was first described in 1837 and it has been recorded many times since. At first it was described only as a postmortem curiosity and for a long time it has been felt that the symptomatology of adrenal hemorrhage was so obscure that there was little probability of establishing a symptom complex which would facilitate ante mortem diagnosis and treatment. In recent years, however, attempts have been made to do this, often with success. Two of the more recent contributions on this subject, those by Goldzieher and Gordon and by Arnold have served to emphasize the importance of the disease, the possibility of its clinical recognition, and the curative therapeutic possibilities.

The phenomenon is especially apt to manifest itself in the first few days of life, following the same time range as that of hemorrhage of the newborn, and may be but a part of the picture of this condition, or it may occur following birth trauma, asphyxia, eclampsia, or as a complication of syphilis, hemophilia, extensive burns, diphtheria or embryonic cystic processes. It does occur occasionally at later ages. Goldzieher and Gordon were able to collect reports of 37 cases of adrenal hemorrhage in the newborn and 38 in older persons, in which clinical information was available and to these they added 6 additional cases 5 of which were diagnosed during life, making a total of 81 cases. The lesions varied from small hemorrhages, through diffuse hemorrhagic infarction, to destructive hemorrhages, and massive hemorrhage which occasionally ruptures into the peritoneal cavity.

The important points in the symptomatology as outlined by these authors are high fever, hemorrhagic rash, rapid respiration, cyanosis, convulsions and evidences of internal hemorrhage.

The fever may be a part of a general infection in which adrenal hemorrhage appears as a complication or it may occur in the absence of infection. The explanation offered is that the adrenal medulla participates in the regulation of body tem-

perature by stimulating the sympathetic nervous system, and particularly the thyroid gland, while the cortex checks the heat producing activity of the thyroid. It has been shown that destruction of a sufficiently large proportion of cortical tissue produces pyrexia, provided thyroid function is unimpaired. These authors as well as Arnold however point out that the temperature may remain normal, even in massive hemorrhage, presumably when there has not been too great cortical destruction.

Rapid respiration is sometimes so pronounced a symptom as to give rise to an erroneous diagnosis of pneumonia. The term "pseudo pneumonia of the newborn" has been suggested as descriptive of the phenomenon. Bilateral adrenalectomy in dogs results in rapid respiration and hyperventilation of the lungs. The administration of cortical hormone restores normal respiratory rate. Goldzieher injected cortical hormone in a case of adrenal hemorrhage, reducing the rate from 50 to 31 per minute. The function of the cortical secretion in controlling tissue respiration has been discussed above and presumably the rapid respiration observed in some cases of adrenal hemorrhage is a result of cortical destruction. This symptom is said to be more pronounced in the new born than in older children or adults with adrenal hemorrhage.

Hypoglycemia also occurs, probably due to medullary insufficiency.

Arnold emphasizes the diagnostic importance of the symptoms of internal hemorrhage with collapse, comparable to those observed in ruptured ectopic pregnancy, often with a palpable abdominal tumor in the region of the kidneys, pronounced abdominal distention, sometimes with a bluish discoloration, sometimes a doughy consistency to the abdomen due to intraperitoneal blood, and often pain which may be excruciating. Not infrequently, particularly in massive hemorrhage, the symptoms of shock may be so pronounced as to entirely mask the previously enumerated symptoms referable to endocrine disturbance, as pyrexia and rapid respiration.

Treatment is directed against (a) the internal hemorrhage, (b) the adrenal insufficiency and (c) the hypoglycemia. It has been suggested that the convulsions sometimes observed may be hypoglycemic in origin. As has been shown by Arnold and others prompt transfusion may sometimes be life saving. In those cases in which the hemorrhage is a part of the picture of the hemorrhagic diathesis of the newborn transfusion may be looked upon as curative as well as palliative. The administration of cortical hormone appears logical and has been employed in one case by Goldzieher and Greenwald with beneficial results. Glucose is, of course, indicated to combat the hypoglycemia. While death usually occurs early the illness may last for several days. One patient succumbed on the fortieth day of the illness.

Recognition of the fact that during its involutionary phase directly after birth the adrenal gland is especially subject to hemorrhagic incidents or catastrophes, and knowledge of a fairly clear cut symptom complex should eventuate in more frequent and earlier clinical recognition of suprarenal hemorrhage, with consequent increased incidence of recovery.

—W T V

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## *CLINICAL AND EXPERIMENTAL*

### BACTERIOLOGIC AND SEROLOGIC STUDY OF EIGHTY-NINE CASES OF DYSENTERY IN WHICH *B. DYSENTERIAE FLEXNER* AND *B. DYSENTERIAE SONNE* (*B. DYSENTERIAE* *CASTELLANI-SONNE*) WERE ISOLATED AS THE CAUSATIVE AGENTS\*

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SHIGA in 1897 made the fundamental discovery of a small bacillus as the etologic agent in dysentery. That year, during the progress of an epidemic in Japan, he isolated a short rod with rounded ends from the excreta, as well as from the mesenteric glands, of thirty-six fatal dysentery infections. This investigator<sup>64</sup> was undoubtedly the pioneer to resort to the use of serologic methods for the detection and identification of the causative agent of a disease. Literally working backwards, he regularly found in the feces of the afflicted an organism which, when cultured, was agglutinated by the blood serum of dysentery patients, while agglutination did not occur with (1) the serum of normal healthy individuals, (2) the blood serum of any patients affected by diseases other than dysentery, and (3) commercial antiserum prepared with unrelated organisms. He also demonstrated that human blood serum was capable of reactivating inactive antidysenteric horse-serum, and believed that the antidysenteric serum would find the proper complement when injected into the human body.<sup>65</sup> Shiga was undoubtedly the first to effect the immunization of horses by the subcutaneous injection of a saline suspension of killed dysentery bacilli which had been grown on agar.<sup>66</sup>

During the past three decades, because of the prevalence of this infection in

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various countries, the isolation of similar, but serologically unrelated organisms has been made by many investigators. Chief among these workers have been the following: Flexner,<sup>24</sup> Strong and Musgrave<sup>25</sup> working in Manila in 1900, Kruse<sup>43</sup> in Germany in 1900, Park<sup>47</sup> at Seal Harbor, Mt. Desert, Maine, in 1902, Duval and Bassett<sup>49</sup> at Baltimore in 1902, Duval and Shorei,<sup>51</sup> Wollsten,<sup>9</sup> Kendall,<sup>38</sup> Hiss and Russell<sup>30</sup> in 1903, Sonne<sup>69</sup> in Denmark in 1915, d'Heelle<sup>32</sup> in France in 1916, Øehnell<sup>52</sup> in Sweden in 1918, Mita<sup>18</sup> in Japan in 1921, Hoshi<sup>50</sup> in Manchuria in 1929.

Thjøtta<sup>73</sup> has divided the microorganisms studied by these investigators into three groups. The members of each being independent from the other two (1) in fermentative powers, and (2) in serologic reactions. The identification of these strains as the agents responsible for the major portion of this well recognized clinical condition has repeatedly confirmed Shiga's concept of bacillary dysentery.

A comprehensive review of the literature indicates that invasions with dysentery bacilli have been world-wide in their occurrence, and that epidemics were particularly numerous during the period from 1927 to 1932. Dysentery due specifically to the members of this group designated as *B. dysenteriae* Sonne have been frequently reported following the publication of Sonne in 1915.

During the last five years several cases of sporadic dysentery have occurred in this district. In 1929 during the months of August, September, and October a small epidemic (about forty cases) of infantile dysentery was manifested here. A relatively high percentage of those who were infected and less than one year of age succumbed to the disease. Organisms of the dysentery group identified by cultural and fermentation reactions, were regularly recovered from the patients. As a control during the interval from January to July, 1930, the stools from 100 healthy infants were examined and no members of the dysentery group were isolated from any of the specimens. A similar study by Castellani<sup>5</sup> on normal individuals failed to detect organisms of this group under ordinary conditions.

The present paper is a report of an investigation of another more extensive and somewhat more acute epidemic of infantile dysentery which appeared in this community later in the same year, i. e., from July, 1930 to January, 1931. The incidence of the disease was greatest during the later part of August and throughout the month of September. (It is of interest that an epidemic of infantile diarrhea was present at the same time in Detroit, some 59 miles from Flint. Poole et al.<sup>60</sup> after a limited bacteriologic study were unable to satisfy themselves that members of the dysentery group were responsible for the epidemic.)

Eighty-nine cases were studied. 67 of these were hospitalized, and the remaining 22 were taken care of in their homes. Dysentery bacilli were recovered from 60 of these individuals. It was found that the germs which were isolated during the course of this investigation fell into three groups, and in the following pages they will be referred to as Types I, II, and III, respectively. It is to be noted that this designation does not correspond to the Types I, II, and III of Thjøtta. The following work was carried out to discover if possible the causative agent responsible for this epidemic, and it is believed that this objective was attained.

## METHODS AND APPARATUS

The basic medium was prepared from "Lemco" meat extract. The liquid component was made by adding 5 grams of extract to each liter of water, to this solution was added 1 per cent Difco Bacto Peptone and 0.5 per cent NaCl, the medium was then adjusted with N/1 NaOH to a reaction of  $P_H$  7.6, after which it was boiled and filtered. Agar, 2 per cent, was added to the broth to be used in the preparation of agar plates and slants. The broth and agar mediums were placed in 15 by 150 mm tubes and autoclaved at  $110^{\circ} C$  for twenty minutes. To prepare the plates, the agar medium was liquefied by heat and then cooled to  $45^{\circ} C$  before pouring into Petri dishes, about 12 cc quantities were placed in the dishes and then allowed to solidify, the water of condensation collecting on the surface of the agar was removed by evaporation before the plates were used.

*Glassware*—All glassware was thoroughly cleaned and dried, tubes were plugged with cotton and subjected to dry heat sterilization before being filled with medium. Petri dishes (100  $\times$  15 mm) were wrapped in heavy manila paper and sterilized by dry heat.

*Special Mediums*—For the fermentation tests six differential mediums were employed, glucose, lactose, sucrose, maltose, mannitol and rhamnose. Stock solutions of these substances in 20 per cent strength were prepared in distilled water, from these the final 1 per cent dilutions in broth were made and Andrade's indicator\* was added to detect acid production. The hydrogen ion concentration of all mediums was determined with a Quinhydrone electrode, both before and after sterilization. These solutions were adjusted to a final hydrogen ion concentration of 6.8 with N/1 NaOH. Sterile Durham tubes (100  $\times$  15 mm) containing inverted vials (40  $\times$  10 mm) were then filled one-third. The tubes were subsequently placed in an Arnold sterilizer and heated for twenty minutes on three successive days. After inoculating into the various mediums, as a matter of convenience in reading the tests, the fermentation tubes were placed in open wire racks holding four rows of ten tubes each.

*Litmus Milk*—The ordinary litmus milk was employed. A concentrated solution of litmus was added sufficient to color the milk a deep blue. This was sterilized by the fractional method.

*Agglutination Technique*—In the serologic tests, tubes (75  $\times$  10 mm) of resistant glass were used. Copper racks containing three rows of ten holes each are convenient. Sterile graduated pipettes were selected in making the serum dilutions and in delivering the antigen suspensions. The antigens were grown on plain agar slants for eighteen hours. They were then washed with normal saline containing 0.4 per cent formaldehyde. The antigen suspensions were then filtered through sterile filter paper and made up to a standard density. The immune serums, Shiga, Hiss and Flexner, were commercial preparations,† and the Sonne immune serum was kindly supplied by R. L. Nelson. The serum dilutions (from 1:80 to 1:20,000) were prepared in sterile tubes (150  $\times$  15 mm) by the addition of saline. It is to be noted that these dilutions were used within thirty minutes after they were prepared.

\* Andrade's Indicator was used in special fermentation reactions by Large in India, Ferry and Bensted in Egypt, Johnston and Brown in Canada and Welch and Wickle in the United States.

† These serums were obtained from Parke Davis & Company.

The antigen suspension was added in 0.5 cc quantities to 0.5 cc volumes of the serum dilutions which had been placed in ten small test tubes (75×10 mm). The tubes were then thoroughly mixed and shaken for two to three minutes, after which they were incubated at 37° C for eighteen hours and the readings noted. A series of control tubes were made with the pedigreed antigens and studied simultaneously with the antigen suspensions of the organism isolated from the patient. The maximum titer of Type I, II, and III as well as the control antigens, are presented in Table II. Frequently there was no agglutination with the antigen in low dilutions of the homologous serum, while with the same serum in higher dilutions the antigen did give agglutination, the well known proagglutinoïd zone phenomenon.

#### ISOLATION AND IDENTIFICATION OF ORGANISMS

Stool specimens were brought as soon as obtainable to the laboratory. Approximately 3 gm of the suspected material was transferred into tubes (100×15 mm) containing about 5 cc of sterile broth. The suspensions were thoroughly mixed by rotating the tubes between the palms of the hands for three minutes. These were then allowed to settle out at room temperature for fifteen minutes, thus giving a suspension that was free from large particles for preparing the dilutions.

A platinum loop (2 mm) was used to transfer samples of the supernatant material. Two dilutions were made from each specimen as follows. Dilution 1 contained the 3 grams of infected material suspended in 5 cc of broth, from this Dilution 2 was prepared by transferring 3 loopfuls of Dilution 1 into 2 cc of sterile broth. A loopful of suspension was taken from each tube and placed upon the surface of a solidified agar plate and then spread by means of a sterile bent glass rod. By this method, following incubation the desired number of colonies, 25 to 100, was usually obtained on each plate.

The study of the growth appearing on the agar surface proved to be extremely interesting because of the variety of colonies present. It is noteworthy that plain agar medium<sup>34</sup> was used and gave excellent results in the differential study of the colonies, however we ordinarily select eosin-methylene-blue or Endo's medium for this type of work because of the additional aids for identification. Observations were made after sixteen hours' incubation, the colonies being first examined by transmitted light, then against a black background (an improvised board covered with black paper). An ordinary hand lens was employed in this examination. Isolated colonies were marked and reexamined microscopically with the low power lens. Material was transferred with a platinum wire from the well separated colonies into lactose broth contained in fermentation tubes, and then incubated overnight. The preliminary use of this medium eliminated all lactose-fermenting strains. A loopful of the culture was removed from those tubes in which there was no acid or gas production and examined both in hanging drop and in stained preparations. Information regarding the size, shape, and structure of the cell was obtained in this manner and confirmed by the Gram stain. Those organisms which showed no acid or gas production in lactose broth were then inoculated into the following mediums

glucose, lactose, sucrose, maltose, mannitol, and rhamnose contained in Durham tubes, after inoculation, they were incubated at 37° C and readings were made at twenty-four-hour intervals for ninety six hours. Controls with each series were made concomitantly both with stock laboratory strains and uninoculated tubes (to check the indicator) on all mediums employed (Table I). From the action of the organisms on the special mediums, they were divided into three distinct types. These special mediums although not as reliable as serologic tests are invaluable as a rapid diagnostic method for distinguishing between the various strains. "In fact, as justly remarked by Morgan, more varieties can be differentiated in this way than by using merely biological methods."<sup>10</sup> In order to have material available for further study, sub cultures were made on plain agar before discarding the fermentation tests.

After the epidemic, during the months of December and January, the fermentation tests were repeated. The same routine was employed as in the original procedure, excepting that the readings were taken rather frequently (every twenty-four hours) for a period of three weeks. This method was used for the purpose of noting any delayed fermentation. Type II, which was isolated from seventeen cases, showed latent fermentation of lactose up to seven days. Characteristic readings were usually obtained with the other two types at the end of twenty-four hours (Table I).

TABLE I  
COMPARATIVE DIFFERENTIAL REACTIONS OF DYSENTERY ORGANISMS

TYPE	NO OF CASES	GLUCOSE 96 HR	LACTOSE 96 HR	SUCROSE 96 HR	MALTOSE 96 HR	MAN- NITOL 96 HR	PHAM- NOSE 96 HR	LITMUS MILK 96 HR
Type I	26	A*	-	-	A	A	A	AC
Type II	17	A	A <sup>1</sup>	A	A	A	A	A
Type III	17	G <sup>2</sup>	-	G	G	-	-	K
CONTROLS								
Shiga <sup>3</sup>		A	-	-	-	-	-	AK
Hiss		A	-	-	A	A	-	AK
Flexner		A	-	A	A	A	A	K
Sonne <sup>4</sup>		A	A	A	A	A	A	A
Metadysentericus A <sup>5</sup>		A	A	A	A	A	-	AC
Ceylonensis A (A D)		A	A	A	A	A	A	AC
Ceylonensis A (S H)		A	A	A	A	A	A	AC
Ceylonensis A (P D)		A	A	A	A	A	A	AC
Ceylonensis B (R W)		A	A <sup>1</sup>	-	A	A	A	AC
Shiga <sup>3</sup>		A	-	-	-	-	-	AK
Hiss		A	-	-	A	A	A	AK
Flexner V		A	-	-	A	A	-	AK
Flexner W		A	-	-	-	A	-	K
Flexner X		A	-	-	A	A	-	K
Flexner Y		A	-	-	A	A	-	K
Flexner Z		A	-	-	A	A	A	K

\*A acid, A, variable (acid or negative), G acid and gas, AC acid and clotted, K, alkaline, AK acid to alkaline.

<sup>1</sup>Tubes were frequently observed ultimately (end of 168 hours) all produced acid in lactose.

<sup>2</sup>Tubes from four of this Type repeatedly produced gas in sucrose and maltose as well as in glucose.

<sup>3</sup>These strains were received from American Type Culture Company.

<sup>4</sup>This strain was received from Nelson R. L.

<sup>5</sup>These strains were received from Sir Aldo Castellani.

These strains were received from Parke Davis & Company.

## CULTURAL AND MORPHOLOGIC CHARACTERISTICS

*Method*—Plain agar plates were prepared and when solid and free from water of condensation a loopful of a young broth culture was transferred to the center of the plate and subsequently spread with a sterile bent glass rod. After sixteen to eighteen hours incubation at 37° C an examination of the plates showed the presence of three distinctly different varieties of colonies. It was extremely interesting to note that all of the strains belonging to any one of the three types based on the fermentation reactions produced similar colonies, therefore colony appearance was an additional means of separating the strains. The cultures were placed into three groups—Type I, II and III on the basis of fermentation reaction, colony type and serologic response.

*Type I*—All plates streaked with suspension of organisms belonging to this type showed on macroscopic examination round glistening, moist translucent

TABLE II

DIFFERENTIAL AGGLUTINATION REACTIONS OF DYSENTERY ORGANISMS WITH IMMUNE SERUMS

ORGANISM	NO OF CASES	SHIGA ANTISERUM	NO OF CASES	HISS ANTISERUM	NO OF CASES	FLEXNER ANTISERUM	NO OF CASES	SONNE ANTISERUM
Type I	1	— <sup>1</sup>	1	100	1	400	1	—
	5	—	5	—	5	1,000	5	—
	2	—	2	—	2	2,000	2	—
	2	—	2	—	2	5,000	2	—
	2	—	2	100	2	5,000	2	—
	2	—	2	200	2	5,000	2	—
	1	—	1	2,000	1	5,000	1	—
	2	200	2	—	2	5,000	2	—
	1	400	1	100	1	5,000	1	—
	3	—	3	100	3	10,000	3	—
	2	—	2	200	2	10,000	2	—
	1	—	1	1,000	1	10,000	1	—
	2	400	2	200	2	10,000	2	—
Type II	17	—	17	—	17	400	17	2,000
Type III	17	—	17	—	14 3	— 400 <sup>2</sup>	17	—
CONTROLS								
Shiga <sup>3</sup>		5,000		—		100		100
Hiss		—		200		1,000		—
Flexner		100		—		5,000		—
Sonne <sup>4</sup>		—		—		400		2,000
Metadysentericus A <sup>5</sup>		—		—		400		1,000
Ceylonensis A (AD)		—		—		400		2,000
Ceylonensis A (SH)		—		—		400		1,000
Ceylonensis A (PD)		—		—		400		2,000
Shiga <sup>4</sup>		2,000		—		—		—
Hiss		—		200		—		—
Flexner V		200		100		2,000		—
Flexner W		—		—		5,000		—
Flexner X		—		100		1,000		—
Flexner Y		80		200		10,000		—
Flexner Z		100		400		10,000		—

<sup>1</sup>The titer is the highest dilution of the serum which caused complete or nearly complete agglutination of bacteria (3+ or better) — indicates negative

Type I was later isolated from these three cases

<sup>2</sup>Numbers 3, 4, 5, 6 See footnote Table I



bluish-white colonies of from 2 to 4 mm in diameter. Upon further incubation the colonies increased in size. However, the edge was no longer regular, but became crenated. Microscopic examination after sixteen hours' growth showed short rods with slightly rounded ends, and these were decolorized by Gram's method. The average size of the cells was 1 to 3 microns in length and 0.5 to 0.8 microns in breadth. The organisms obtained both from the broth cultures and from the colonies on the agar plates were nonmotile. The general appearance of the colonies, the morphologic appearance of the bacterium, the fermentation reaction and agglutination results when compared with the controls and the published data of other workers indicated that this type belonged to the Flexner group (Tables I and II).

Organisms belonging to this type were recovered from twenty-six of the infections. Eight deaths occurred among the children under two years of age in this group. Type III, to be described later, was simultaneously isolated from four of these cases which had terminated fatally.

*Type II*—These strains, Tables I and II, were identical in their fermentative and agglutinative reactions with the control cultures of *B. dysenteriae* Sonne. After sixteen hours' incubation the colonies were found to be round, smooth edged, slightly opaque and moist, with a bluish tinge, and varied in size from 0.5 to 2.0 mm. On continued incubation all the colonies usually acquired a definite blue tinge, the surface being somewhat convex and the margin smooth and regular. On rare occasions larger colonies with irregular borders suggestive of R forms were observed but subcultures from these colonies gave the typical smooth S form. Microscopic examinations showed nonmotile, gram-negative rods varying from 0.8 to 2 microns in length and from 0.4 to 0.6 microns in breadth. Occasional involution forms were observed. Identification of these strains by their early production of acid in rhamnose and latent fermentation of lactose (three to seven days) classify them as *B. dysenteriae* Sonne (Table I). Serologic tests with this group are also comparable (Table II). Attention is called to the fact that Type II corresponds in its fermentation and serologic reactions with the strains of the *Meta-dysenteriae* organisms of Castellani (Tables I and II).

*Clinical Observations*—This organism was isolated from seventeen cases, chiefly in children between one and seven years of age. In addition, four of these strains were recovered from the same number of nurses that had previously been on duty in the ward reserved for colitis patients. Moreover, in each of four families wherein there were two members attacked, the identical organism was isolated from each of the individuals. Indeed, it was noted that the second infection in the same family occurred approximately a week after the onset of the first. The microorganisms which are included in this group always caused conditions in which the clinical manifestations were sudden and severe. Bloody, mucous stools were passed frequently (6 to 15 a day), accompanied by a rapid elevation in temperature ( $102^{\circ}$  to  $105^{\circ}$ ). The blood count, which was not characteristic, varied from 12,000 to 18,000 white blood cells, with a corresponding increase in polymorphonuclear leucocytes and a decrease in lymphocytes. These symptoms usually ceased in seventy-two to ninety-six hours. No deaths were associated with the members of this group of organisms.

*Type III*—The colonies on agar plates showed considerable variation in

size They were round and convex, with a glossy mucoid appearance On continued incubation the colonies increased in size and transparency, often coalescing to form a viscous mass It would seem that the members of this group are mucoid variants<sup>23</sup> of Type I since they fail to agglutinate in the presence of the antisera of Shiga, Hiss, Flexner, and Sonne as well as homologous antisera This fact is in agreement with our observations<sup>2</sup> on the mucoid variants of *B. paratyphosus* A, *B. psittacosis*, *B. ictteroides* and *B. coli*, however, we have been unable to dissociate these mucoid forms to S forms giving the characteristic reactions of the Flexner bacillus The individual cells were actively motile, short, and plump, gram-negative rods The growth of the members of this group in lactose broth was accompanied by the production of acid and gas (Table I) There was no agglutinative ability observed with any of the immune sera when used with these strains (Table II) The colonial growth was accompanied by a distinctly obnoxious, putrid odor

*Clinical Observations*—Strains belonging to this type were found in seventeen of the patients In four of the fatal infections Type I was also present There were five deaths in this group four of which were associated with other conditions, as follows three with malnutrition, and one with bronchopneumonia The deaths occurred in infants less than one year of age

#### ANALYSIS OF TABLES

In Table I may be found the reaction of all the cultures and controls on the special mediums studied The strains of Type I show the characteristic fermentative reactions typical for the Flexner bacillus and were considered to be identical with this germ The organisms gave the following reactions in the special mediums used acid in glucose and mannitol, but variable in maltose and rhamnose, no change occurred in lactose and sucrose Litmus milk became acid and coagulated The fermentation reactions of Type II are seen to be identical with *B. dysenteriae* Sonne Nine of these strains produced acid in lactose after four days, while eight did not produce acid until the sixth day The reactions of the Meta-dysenteriae cultures obtained from Sir Aldo Castellani are identical with the fermentative reactions of *B. dysenteriae* Sonne This suggests that the Meta-dysenteriae of Castellani, the present Type II and *B. dysenteriae* Sonne are closely related organisms However the colony formation of the Meta-dysenteriae of Castellani closely resemble the variant mucoid form Type III Type III fermentation reactions are identical with those given by typical strains of the Morgan bacillus<sup>19</sup>

Upon reference, in Table II are given the maximum agglutination titers of the Flexner serum with strains of the three types isolated as well as with pedigreed cultures Type I was isolated from eight of the fatal infections which gave the following agglutination results

#### TYPE I

3 strains agglutinated Flexner Immune Serum 1	10,000
2 strains agglutinated Flexner Immune Serum 1	5,000
1 strain agglutinated Flexner Immune Serum 1	2,000
1 strain agglutinated Flexner Immune Serum 1	1,000
1 strain agglutinated Flexner Immune Serum 1	400

\*Soule, M H, with Heyman Anne M (In press)

Type II was agglutinated by *B. dysenteriae* Sonne immune serum 1:2,000 and by Flexner immune serum 1:400. In a similar series of agglutination reactions the Meta dysenteriae of Castellani also gave very comparable results. From the results obtained by the use of the Meta dysenteriae cultures as controls in our investigation the biochemical and the serologic reactions, which are given in Tables I and II, indicate that these organisms are identical with *B. dysenteriae* Sonne, isolated by Sonne in Denmark in 1915. This fact is also in accordance with the results obtained by Cerutti in a report by Castellani<sup>12</sup> and by Koser et al.<sup>13</sup> If we are to accept the criteria established by Shiga (i.e. the identification of microorganisms by serologic methods), we must recognize that the organism discovered by Sonne in 1915, and classified by Thygesen as Type III, is the same organism found by Castellani in Ceylon in 1904 and in 1905.<sup>6, 7</sup> It is suggested that this organism be reclassified as *B. dysenteriae* Castellani-Sonne. Investigations by Cerutti<sup>14</sup> confirm this opinion.

It is not clear as to whether the cultures used by Koser et al.<sup>13</sup> under the designation of Ceylonensis A and Ceylonensis B are the same strains included in the present study under the same names. Our strains were kindly furnished by Sir Aldo Castellani as already indicated. Differences in the fermentative power of Ceylonensis A and Ceylonensis B similar to those observed by Koser were noted indicative of the fact that they do not belong to the same group of organisms and the terminology is therefore confusing.

The serologic reactions of Type III were unsatisfactory, only three strains showed agglutination by Flexner immune serum at a dilution of 1:400. From the patients infected with those three strains Type I was later isolated. Morgan and Ledingham<sup>10</sup> found that the agglutination results of *B. morganii* were not specific. Kligler<sup>10</sup> in a study of seventeen strains of *B. morganii* found them culturally identical but highly diversified antigenically. "While no conclusions can be drawn regarding the pathogenic significance of this bacillus, the wide diversities of antigenic properties raise the question as to the specific relationship of the various cultures met with as well as their relation to a definite class of pathologic processes in man."

The question frequently arose as to whether or not the strains placed in Type III were *B. coli*. The cultures were carefully examined with this consideration in mind but all of the data indicated that such was not the case. It would have been very desirable to compare the reactions of these strains simultaneously with known cultures of *B. morganii*, but such experiments were not carried out.

In Table III are summarized the clinical data of the 89 cases of dysentery.

TABLE III  
CLINICAL DATA OF DYSENTERY

NUMBER OF CASES	AGE	DEATHS
64	6 weeks to 2 years	17
20	2 years to 8 years	0
4	18 years to 21 years*	0
1	27 years**	0

\*Student Nurses with Type II (Sonne)

\*\*Mother of Infant with Type III (Unclassified)

studied Although there were 17 deaths, 7 of these were associated with other diseases Hospital charts were carefully examined An increase in temperature was noted in every instance, the maximum curve in each case ranging from 99.6° to 105° The blood count was not indicative, although there usually appeared an increase in the white blood cell count which was accompanied by an increase in the polymorphonuclear leucocytes and a decrease in the lymphocytes The red blood cell count appeared normal, excepting in cases of extreme dehydration The stool specimens obtained varied from four up to twenty per day Some contained blood and mucus while others were watery, green, with small amounts of mucus Vomiting occurred in about 50 per cent of these cases The average length of illness was approximately fourteen days The aforementioned data were gathered from the 67 infections which were hospitalized

#### DISCUSSION

*Type I*—Type I, which is seen to agree serologically with B dysenteriae Flexneri, was found most frequently in this epidemic Bacteriologic, fermentative, and serologic identification of this type was obtained in 26 infections and 8 of these proved fatal In 3 of the fatal infections this germ was isolated from the mesenteric glands at postmortem examination Duval and Bassett<sup>29</sup> also obtained this organism from the mesenteric glands and liver of infants after death, and Kinloch<sup>39</sup> in Scotland also obtained the Flexner bacillus from the intestine of a two-year-old child at autopsy Fatal infections by this microorganism, especially in children have been reported by the following investigators Flexner<sup>22</sup> Duval and Bassett,<sup>29</sup> Park,<sup>47</sup> Wollstein,<sup>79</sup> Davison,<sup>18</sup> and Silverman<sup>67</sup> in the United States, Rajchman and Western<sup>41</sup> in the Eastern Mediterranean District, Matira<sup>46</sup> in the Jharia Mining Settlement in India, Kinloch<sup>39</sup> (1919) in Scotland, Webster and Williams<sup>77</sup> in Australia, Warren<sup>4</sup> in England, Rosenbaum<sup>63</sup> and Rimpau<sup>62</sup> in Germany, Anche and Campana<sup>3</sup> in France, Torres Umaña<sup>7</sup> of Bogota, Padua<sup>46</sup> in the Philippines Although infections with these organisms are usually associated with infancy, some of the cases reported by the above investigators have been in adults Wollstein,<sup>79</sup> in a report of 39 positive infections of infantile diarrhea in 1903, in which 29 infants died, found that the causative agent agglutinated with Flexner immune serum in a dilution from 1:50 to 1:3,000 Again, in a second study, in 1922,<sup>80</sup> of 86 infections, 20 of which were positive she found that the agglutination reactions of the mannite-fermenting strains, which were isolated, to be high with Flexner polyvalent serum, i. e., 1:3,200 to 1:6,400 The significant factor in these results is that the highest agglutination titer was obtained with polyvalent serum Manifold and DeMonte<sup>47</sup> investigating outbreaks of dysentery among the troops in India, isolated B flexneri as a causative agent The agglutination results obtained by these workers with stock laboratory Flexner immune serums against their Flexner bacilli varied from dilutions of 1:500 to 1:5,000 The data obtained with their polyvalent immune serums are comparable with the serologic results given by our Type I with Flexner polyvalent immune serum, which varied from 1:400 to 1:10,000 (Table II)

The wide distribution of this organism and its association with a high mortality rate in children is worthy of note

*Type II*—Strains of this type which were isolated from seventeen of the dysentery infections, possess the morphologic, cultural, fermentation and serologic characteristics of *B. dysenteriae* Sonne and excepting colony type the *Meta-dysenteriae* of Castellani. Sonne<sup>20</sup> in 1915 by repeating the classical serologic experiments of Shiga, was the first to demonstrate that this type was serologically specific and independent of other bacilli of the dysentery group. He also showed that this was the causative agent in a series of infections in Denmark.<sup>21</sup> d'Herelle,<sup>22</sup> without the knowledge of Sonne's work, isolated and described the same organism again in 1916. Since then this microbe has been found by the following workers in several countries: Thjøtta<sup>23</sup> in Norway, Øhnell<sup>24</sup> in Sweden, Mita<sup>25</sup> and Kobayashi<sup>26</sup> et al in Japan, Patterson and Williams<sup>27</sup> in Australia, Andrewes,<sup>28</sup> Nabarro,<sup>29</sup> Smith,<sup>30</sup> Channon,<sup>31</sup> Evans,<sup>32</sup> Clayton and Hunter,<sup>33</sup> Clayton and Warren,<sup>34</sup> Cann and Novasquez<sup>35</sup> in England, Fraser, Kinloch and Smith,<sup>36</sup> Fyfe,<sup>37</sup> Hay<sup>38</sup> in Scotland, Perry and Bensted<sup>39</sup> in Egypt, Large<sup>40</sup> in India, Buchanan and Roux<sup>41</sup> in South Africa, Johnston and Brown<sup>42</sup> in Canada, Gilbert and Coleman,<sup>43</sup> Nelson,<sup>44</sup> and Welch and Mickle<sup>45</sup> in the United States.

Duval and Shorer,<sup>46</sup> and Kendall<sup>47</sup> in 1904 noted atypical fermentation reaction, i. e., slow-lactose fermenting organisms, which also gave a low agglutination reaction with polyvalent immune Flexner serum. The organisms giving these reactions were classified as atypical Flexner bacilli. It is conceivable that these strains are the same organism as that identified by Sonne in 1915. However, in 1904 and 1905 Castellani isolated two organisms at Ceylon<sup>48</sup> which he described in 1907 under the names *B. ceylonensis* A and B, *ceylonensis* B, the former being very similar to the Sonne bacillus. In 1927<sup>49</sup> and 1932<sup>50, 51</sup> Castellani classified these two organisms and other closely related forms as "*Meta-dysenteriae* Bacilli," to identify those members of the dysentery group which produce acid slowly in lactose. The cultures of *Meta-dysenteriae* received from Castellani, and used as controls in this investigation, have proved to be identical in their fermentation and serologic reactions with *B. dysenteriae* Sonne obtained from Nelson (Tables I and II).

It is to be noted that the members of this group in contrast to infections with Flexner's bacillus were never, of themselves, able to cause the death of the patient. However, Evans<sup>32</sup> in England and Hay<sup>31</sup> in Glasgow report *B. dysenteriae* Sonne isolated as the causative agent in three fatal cases. It is indeed interesting that most workers have isolated this microorganism from children between one and ten years of age. In the present epidemic, as noted, Sonne infections occurred chiefly in children less than eight years of age, and none of these terminated fatally. Typical photographs of the colonial growth of this organism may be found in articles by Thjøtta,<sup>23</sup> Kobayashi et al,<sup>26</sup> Cann and Novasquez<sup>35</sup> and Welch and Mickle.<sup>45</sup> The characteristic delayed fermentation of lactose serves admirably as a means of identifying this organism from other members of the dysentery group.

Since Shiga's classical agglutination studies, the clumping of an organism by its specific antiserum has been held as a final criterion in the identity of the microorganism. In reviewing the literature it was noted by Gilbert and Coleman<sup>43</sup> that *B. dysenteriae* Sonne gave agglutination in low dilutions with poly-

valent immune dysentery serum Patterson and Williams<sup>8</sup> in Australia found that the reactions with Flexner polyvalent immune serum versus the Sonne bacillus gave agglutination of 1:400, while with the homologous serum, they obtained agglutination in dilutions of 1:2,400. Perry and Bensted<sup>9</sup> working in Egypt have reported the following "In the serological investigation of the strains isolated, a few of the organisms were agglutinated to a low titer by a polyvalent serum prepared in these laboratories from type strains of *B. dysenteriae* Flexner V, W, X, Y, and Z." And further, "When titrated against a Sonne serum prepared from classical strains of this organism that had originally been obtained from the National Collection of Type Cultures, all the strains recovered were agglutinated to full titer." The fact that the Flexner species possesses antigenic relationship with many other species has been shown by Andrewes and Inman (1919). Clayton and Warren<sup>17</sup> (1927), confirmed this antigenic relationship which was originally demonstrated by Andrewes and Inman. Gilbert and Coleman<sup>18</sup> (1929), reported the following "In polyvalent dysentery immune serum prepared with the non-lactose fermenting strains, cultures of the Sonne dysentery bacillus are usually agglutinated only in low dilutions." The strains of Type II, isolated in the present study confirm the observations of these investigators, suspensions of the organisms were found to agglutinate regularly with Flexner polyvalent immune serum in a dilution of 1:400 (Table II). Large<sup>14</sup> in the Lahore District in India reported that Type A Sonne will agglutinate in Sonne serum in high titer, Nabarro<sup>15</sup> in England, Johnston and Brown<sup>17</sup> in Canada, Welch and Mickle<sup>18</sup> in the United States reported agglutination from 1:1,280 to 1:2,560 on most occasions with homologous serums in infections from which the Sonne bacillus was isolated. Strains of Type II in the present studies gave an agglutination with Sonne immune serum of 1:2,000 (Table II). Hay<sup>21</sup> reported an agglutination of 1:1,600. The serologic results obtained both with polyvalent Flexner serum and the homologous Sonne serum by these workers are comparable with those obtained with strains of Type II (Table II) from seventeen infections of dysentery. Attention is likewise called to the results given by Welch and Mickle,<sup>18</sup> since they also employed Sonne immune serum from Nelson. These workers report that the *Meta-dysenteroides* strain obtained from the American Type Culture Collection was agglutinated by the Sonne immune serum from Nelson in high dilutions, i. e., 1:2,560. The specific serologic reactions of the *Meta-dysenteroides* strain suggest the identical relationship between this group and *B. dysenteriae* Sonne. The reports of many investigators show that in infections from which *B. dysenteriae* Sonne have been obtained suspensions of the organisms gave, in many instances, a low agglutination titer with polyvalent Flexner immune serums, while with homologous serums the agglutination titer varied from 1:1,280 to 1:2,560. In the present study the *Meta-dysenteriae* cultures of Castellani were agglutinated by Nelson's Sonne immune serum as follows. Two cultures 1:1,000, and two cultures 1:2,000 (Table II). Cerruti<sup>14</sup> in 1930 observed the agglutination of these same strains from Castellani by Sonne serum.

Nelson,<sup>14</sup> Koser et al.<sup>15</sup> prepared immune serums having an agglutination titer of over 1:5,000 when used with strains of the Sonne organism obtained from various sources. These serologic tests confirm Sonne's researches which demon-

strated his organism as having a very definite serologic specificity. The fermentation and agglutination reactions obtained with the strains of Type II identify them as the Sonne bacillus and as is indicated were isolated from seventeen of the infections.

The recent serologic studies of Thjotta and Waaler<sup>14</sup> on the S and R forms of dysentery bacilli Type III have an important bearing on the identification of this organism by serologic methods. In their investigations these workers found that the dissociants were agglutinated by their homologous antiserums at significant titers (1:640) but that the organisms in the presence of the heterologous serums were not agglutinated at titers possessing diagnostic value (1:40). It is also of interest to record that in no instance during the present study were so called R forms of the dysentery bacillus encountered although their possible presence was always borne in mind when examining the plates.

*Type III*—Type III remains unclassified. Although the fermentation reactions of these strains were consistent with those reported for the Morgan bacillus by other workers no serologic relationship with any of the antisera employed could be demonstrated. Morgan and Ledingham<sup>15</sup> in 1909 reported that the agglutination results of *B. morgani* isolated in 1906 are not specific. Kligler<sup>16</sup> in a study of seventeen strains of *B. morgani* found they were culturally identical, but antigenically they were highly diversified. "While no conclusions can be drawn regarding the pathogenic significance of this bacillus the wide diversities of antigenic properties raise the question as to the specific relationship of the various cultures met with as well as their relation to a definite class of pathologic processes in man." Havens and Ridgway<sup>17</sup> in 1929 refer to the Biologic Relationship of Morgan's Bacillus as follows: "While it is not clear that agglutination differences observed between strains of Morgan's bacillus can be explained on the basis of mutation, the results are roughly comparable to those obtained with certain strains of *B. typhosus*, which showed agglutination differences but were identical in complement fixing properties." "Complement fixation reactions between ten strains of *B. morgani* revealed close antigenic identity. The serologic unity demonstrated by this method permitted a study of antigenic relationship of *B. morgani* to members of the typhoid-dysentery group. Close relationship was indicated to the paratyphoid and paradyntery strains studied." Indeed, MacKenzie and Batt<sup>18</sup> found in an antigenic analysis of *B. morgani*, which had been isolated in an epidemic of summer diarrhea, that this organism usually was not agglutinated by the polyvalent sera employed. However, they found that *B. morgani* was agglutinated by polyvalent immune horse serum in a dilution of 1:80. This agglutination of *B. morgani* with immune polyvalent serum is much lower than that obtained with microorganisms of the dysentery group under the same condition. They arrived at the conclusion that "B. paradynteriae isolated during the epidemic has been shown to contain an antigenic component which is also present in one of the cultures of *B. Morgani*." However, in a very recent report of Castellani and MacKenzie<sup>19</sup> of nine strains of *B. morgani* No. 1 which were identical in their fermentation reactions, five different serologic types were separated. They found moreover, that the serum prepared from the culture of the organism, by intravenous injections into the ears of rabbits, agglutinated its homologous organism in most cases 1:800 and 1:1,600.

In the present investigation of the nonagglutinability of strains of Type III (Table II) by the immune serums employed it is suggested that these strains may be variants of Type I or Type II. However, the fermentation and serologic results with strains of Type III are comparable with those reported by MacKenzie and Batt for *B. moirani*.

#### SUMMARY

1 Eighty-nine cases of an acute epidemic of diarrhoeal disease are reported. Eighty-four of the patients were under eight years of age and of this group, 64 were less than two years old.

2 Organisms belonging to the dysentery group were isolated from sixty of these infections.

3 A detailed study of the morphologic, cultural, fermentative and serologic reactions of the various strains isolated is presented.

4 The cultures on the basis of these reactions are classified into 3 groups.

*Type I* appears to be identical in its fermentative and serologic reactions with *B. dysenteriae* Flexner. This type was found most frequently during the epidemic, occurring in 26 cases, eight of which terminated fatally. *Type II* corresponds, colony formation excepted, to *B. Meta-dysenteriae* Castellani and is identical with *B. dysenteriae* Sonne. Members of this group were isolated from 17 cases in which the clinical manifestations were severe but in contrast to infections with the Flexner type, were not fatal. *Type III*, based on fermentation reactions, the members of this group are very closely related to *B. moirani*. However from the observation that this type was present in four of the fatal infections in which the Flexner organism was the causative agent, it is considered that Type III is a variant of Type I. Considerable experience on the part of the authors with the mucoid variants of the members of the colon-typhoid-dysentery groups of organisms lends further support to this view.

5 The data indicate that there were two definite serologic groups, *B. dysenteriae* Flexner and *B. dysenteriae* Sonne, among the three types isolated and that they were the causative agents of the present epidemic of infantile dysentery.

6 On the basis of priority, it is suggested that the name *B. dysenteriae* Castellani-Sonne be given to the members of the Type III dysentery organism of Thjøtta.

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## STUDIES ON THE ETIOLOGY OF POLIOMYELITIS ISOLATION AND CULTIVATION OF AN ORGANISM AND TRANSMISSION OF THE DISEASE IN MONKEYS\*

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### INTRODUCTION

LESS than fifty years have elapsed since Struempell<sup>1</sup> in Vienna first hunted that poliomyelitis, described as a clinical entity by Jacob Heimer in 1840, was probably an infectious disease. Medin,<sup>2</sup> reporting an epidemic in Sweden in 1889, strengthened the idea that paralysis was but one feature of an acute general infection. The modern conception of the disease was developed only a little over twenty-five years ago by Wickman,<sup>3</sup> who in 1905 contributed a monumental work on that great epidemic in Sweden. His epidemiologic studies also added convincing proof of the infectious nature of poliomyelitis and its communicability.

The numerous pathologic studies beginning with Provost and Vulpian<sup>4</sup> in 1865 and culminating in the classical works of Flexner and his associates<sup>5</sup> gave a clear picture of the course of the disease and its far reaching effects in the animal body.

These newly acquired facts served all the more to stimulate a widespread search for the causative agent. In the attempts that followed there was opened a wide field of investigation from which came a more detailed knowledge of the disease. Signaling the experimental approach to the problem was the first successful transmission of poliomyelitis to monkeys accomplished almost simultaneously in Vienna by Landsteiner and Popper<sup>7</sup> and in the United States by Flexner and Lewis.<sup>8</sup> To the last named, however, belongs the credit for the most fundamental achievement whereby the infection was propagated indefinitely through series of animals by means of direct inoculation of the virus into the brain. For, with regard to such crucial experiments, the European investigators had not been successful. The infecting agent was subsequently found to be capable of passing through the pores of a Berkefeld filter. Flexner and Lewis<sup>9</sup> in 1909 and Landsteiner and Levaditi<sup>10</sup> in the following year thereby added poliomyelitis to the known diseases caused by active agents smaller than ordinary microorganisms, and hence known as filterable viruses.

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## ARTIFICIAL CULTIVATION OF FILTERABLE VIRUSES

Numerous attempts have been made to cultivate viruses in general and that of poliomyelitis in particular. It is not inconsistent with the nature of invisible things that there be some discussion as to their properties or to their existence in fact. Certain filterable viruses, much smaller than ordinary bacteria, are conceded to be in all probability optically immeasurable. Curiously, the greatest amount of discussion hinges about those viruses that are not quite so small, yet are capable of passing through different kinds of filters.

Observations made in the past have led to the formulation of certain principles concerning viruses. Some of these statements are perhaps open to question and in the light of newer bacteriology would appear to merit some revision. It has been said that living tissues or cells are indispensable for the growth of etiologic agents of the nature of filterable viruses. Opposed to this is the fact that viruses, none the less, have remained invisible in living tissue. Given the proper chemical and nutritive basis in a culture medium, it should be possible for virus to multiply in tissue containing nonliving cells. Another moot question has concerned itself with whether or not the viruses multiply intra- or extracellularly. This has led to an involved literature on the so called "inclusion bodies." These are said to be the products of degeneration by some, by others they are considered as the virus itself or as the evidence of its activity, and finally as having no specific characteristic. At the moment although their exact nature is uncertain they are not accepted generally as stages in the life cycle of an organism.

These questions may be answered perhaps by observations that have been made upon filterable forms of bacteria. Any discussion of filterability must entail all phenomena of bacterial growth as well as that of virus multiplication. The more recent experimental studies have indicated the importance of complex metamorphoses and life cycles in the growth and development of organisms hitherto believed to be stable and simple. These observations are but reaffirmations of the well grounded pioneer studies of Lankester,<sup>11</sup> Lohm<sup>12</sup> and others. Pleomorphism has been viewed with much skepticism in matters pertaining to the etiologic significance of microorganisms. The occurrence of metamorphosis and a life cycle has been conceded to protozoal forms but has been denied bacteria and viruses. The newer bacteriology appears to have broken away from this point of view.<sup>13 14 15 16</sup> Noteworthy contributions in the field of filterable viruses have been numerous, among them the more recent reports of Rivers,<sup>17</sup> Zinsser<sup>18</sup> and associates,<sup>19</sup> Cowdry,<sup>20</sup> Olitsky,<sup>21</sup> Goodpasture,<sup>22</sup> Noguchi,<sup>23</sup> Flexner,<sup>24</sup> and Shope.<sup>25</sup>

## EARLIER STUDIES IN THE CULTIVATION OF POLIOMYELITIC VIRUS

The original paper of Flexner and Noguchi\* and the subsequent reports of Amoss,<sup>7</sup> Smilhe<sup>8</sup> and Heist and Kolmer<sup>26</sup> defined the characteristics of the "globoid bodies" cultivated from tissues of the central nervous system from human and experimental sources. Certain definite criteria of the cultures included the essential requirement of an anaerobic culture medium containing a piece of fresh sterile rabbit kidney and ascitic fluid. In respect to pathogenicity it was found that only exceptional cultures were infectious and that saprophytic strains were cultivable with difficulty while parasitic ones were even more refractory. Olitsky,<sup>20</sup> repeating these studies, was unable to obtain satisfactory secondary growths in later subplants and characterized these results as disappointing. In these experiments it should be noted that a Berkefeld V filter was used in the preparation of filtrates from the brain and spinal cord as well as

fragments of these tissues. Next he applied the method of cataphoresis to the contents of the culture tubes and was unable to differentiate "microorganisms" from precipitate. This procedure was based on the previous observations made by Olitsky,<sup>22</sup> that poliomyelitic virus in suspensions of nervous tissue migrated to the anode. The power of such "cultures" to infect and induce experimental poliomyelitis was then studied in monkeys. He employed suspensions of anodic material obtained by cataphoresis of poliomyelitic nervous tissue from a monkey and successfully produced the disease in one animal. In one series of subplants derived from the brain and cord of this infected monkey, materials taken from "cultures" in the seventh to the tenth transplants produced experimental poliomyelitis. Prodigious numbers of "globoid bodies" recovered from as many as twenty to thirty or more tube cultures, were inoculated.

From all the available evidence and the conclusions drawn by Olitsky it would appear that the minute particles did not represent true cultures of the globoid bodies or of any living organism. He has suggested the hypothesis that the virus has been adsorbed to the minute particles seen in these "cultures" and which resemble closely the "globoid bodies" of Flexner and Noguchi. Olitsky has further suggested the possibility of the virus having multiplied in a modified living tissue cell culture.

#### EXPERIMENTAL

In the investigation to be reported we had access to material taken from monkeys that had been infected experimentally with an active strain of poliomyelitis virus obtained from the Rockefeller Institute through the courtesy of Dr. Flexner. Two additional strains were obtained from other institutions and represented original Flexner virus that had been used successfully in the production of experimental poliomyelitis.

*Macacus rhesus* monkeys were inoculated\* intracerebrally with 0.2 to 0.5 c.c. of a 5 per cent saline Berkefeld N filtrate prepared from the brain and cord. After definite paralysis had occurred following a typical clinical picture of the disease, the animals were etherized and the brain and spinal cord removed with strict sterile precautions. The tissues were tested for bacterial growth before and after storage in sterile 50 per cent glycerol. This glycerolated material in the primary cultivation experiments was used for inoculation of media after five to six months' preservation in the ice chest at a temperature of 4° C. In the recultivation experiments the materials prepared from the brain and cord of monkeys that had been infected by means of cultures were inoculated into media on the same or on the following day. Such tissues had been preserved in glycerol at ice box temperature for periods ranging from four hours to one to four days, and a maximum period of fifty-four days (Monkey 38).

Culture media was inoculated with 0.1 to 1.0 c.c. of a Berkefeld N filtrate of a 5 per cent suspension of the brain and cord for primary cultivation. Both the filtrates and suspensions separately of brain and cord, respectively, in amounts of 1.0 c.c. were used in the recultivation experiments from monkeys successfully infected with cultures.

#### CULTIVATION

Seven different strains of poliomyelitis virus were studied. "EB" medium to be described later was used as the original substrate from which subplants were made to the "VB" medium. The first cultures were successfully isolated according to the procedure to be reported in the text. For the recultivation of organisms

\*All inoculations in monkeys were performed under ether anesthesia and with strictest surgical asepsis.

†For revised technique and details refer to *Proc. Soc. Exper. Biol. & Med.* 30:92, 1932.

from monkeys that had been successfully inoculated with the cultures it was found that the "VB" substrate alone seemed to be satisfactory

The culture medium consisted of sterilized minced sheep brain contained in a veal infusion broth free from peptone. It was thought highly desirable to avoid the use of fresh living tissue and ascitic fluid such as characterize the Noguchi medium or its modifications. Another departure from the usual technique concerned the oxygen requirements during incubation. No attempt was made to obtain strict anaerobiosis by means of the recognized methods. The reduced oxygen tension and anaerobiosis developed in the medium were found satisfactory. The use of brain tissue suggested itself as a suitable culture medium for a virus that is known to exhibit a marked affinity for the nervous system.

Minced brain medium which will be referred to as VB was prepared in the following manner:

The blood and membranes were carefully removed at autopsy from fresh sheep brain. One kilogram of tissue was added to one liter of veal infusion broth,  $P_{H}$  8.0, free from peptone. The mixture was coagulated thoroughly by boiling for two to three minutes and 0.2 per cent glucose by volume was added. The cooled material was then passed two or three times through a meat chopper. The culture medium was sterilized in small flasks at 15 pounds' pressure for twenty minutes and stored. Test tubes measuring 15 by 15 cm. were filled to a depth of approximately 6 cm. (or about 10 c.c. by volume) and sterilized in the autoclave at 15 pounds' pressure for twenty minutes. The tubed medium was sterilized a second time at 15 pounds' pressure for ten minutes. The final  $P_{H}$  was adjusted (if necessary) to 7.6. This reaction underwent no appreciable change in the course of prolonged incubation of the cultures in the medium owing to its natural buffering quality.

The following experiment was made to determine the degree of heat penetration necessary for proper sterilization, and to rule out the possibility of living tissue in the culture medium.

A maximum recording thermometer was placed in the center of the medium contained in a test tube and held firmly in position 2 cm. from the base. The pressure within the autoclave was raised to 15 pounds and within five minutes the temperature registered  $118^{\circ}\text{C}$ . After exposure of three minutes to this temperature, the steam was shut off and the autoclave opened two minutes later. The temperature within the tube of medium, at this time, registered  $96^{\circ}\text{C}$ . The tube was allowed to cool spontaneously, and thirty five minutes after the beginning of the experiment the temperature within the tissue mass registered  $68^{\circ}\text{C}$ . From this experiment it is evident that an exposure of fifteen to twenty minutes at 15 pounds' pressure would be sufficient to destroy any living tissue and to effect complete sterilization. Resistance of nervous tissue to high temperatures is considerably less than that of other tissues. Pincus and Fischer<sup>32</sup> found them to be extremely labile following exposure of a few minutes to 60 or  $67^{\circ}\text{C}$ .

Tests for sterility were made by incubating the medium at room temperature ( $20^{\circ}$  to  $24^{\circ}\text{C}$ ) and at  $37.5^{\circ}$  to  $38^{\circ}\text{C}$  for three or four weeks. As an additional precaution 3 or 5 c.c. samples of the medium were transplanted to simple and enriched culture mediums and carefully observed for three or four weeks to rule out all possible sources of contamination.

The inoculum, consisting of either filtrates or suspensions as already described, was next added to a series of 8 to 10 tubes of culture medium and incubated at  $37.5^{\circ}$  to  $38.0^{\circ}\text{C}$ \*. Two tubes in each set were left unopened during the three or four weeks' period of observation during which samples from the others were carefully withdrawn for subplants every week or ten days by means of a platinum loop or a sterile capillary pipette (approximately 0.1 c.c.) and for film preparations. Control tests included Berkefeld filtrates and suspensions of poliomyelitic monkey brain and cord inoculated into ordinary simple fluid and solid culture mediums, filtrates and suspensions from normal monkey brains and from herpes, encephalitis and neurocytemia, and finally uninoculated "VB" medium.

Subplants from tubes that yielded growth were made every five to ten days. The second and subsequent generations were found to develop satisfactorily within a week. The un-

\*32-34  $\text{C}$  has been found more satisfactory

opened reserve tubes were examined after growth had occurred in the remaining series and were found to contain the identical organisms.

Subplants from the tubes that showed primary growth with the inoculum were made by means of a single 2 mm. platinum loopful for the first three or four transfers. This was done in order to effect many millionfold dilutions of the original virus. At the same time transfers were also made to ordinary brain heart (Bacto) infusion broth and blood agar plates.

A modified technique was also used for cultivating the virus and maintaining culture subplants in parallel series with VB medium. The method of Kendall<sup>24</sup> was followed in part in



Fig 1—Culture medium VB (tubes A and B) and EB (tubes C and D). Positive growth in tubes A and D showing slight turbidity; uninoculated controls B and C remaining clear with sharp line of division between tissue and fluid. The changes due to microbial growth can be seen on the surface of the column of brain tissue immediately beneath the supernatant fluid.

the preparation of granulated sheep brain substrate to be referred to as EB medium. The "K" medium (hog intestine) of Kendall and sheep brain prepared according to his method and used in the manner suggested by him for the "K" substrate gave negative cultivation results in our study. Hence the extraction procedures were modified somewhat and the final concentration of brain tissue in 0.85 per cent sodium chloride was made up to 10.20 per cent by weight instead of 2 per cent. The final  $P_H$  was adjusted to 7.6 instead of 7 to 7.4 and the temperature of incubation was 37.5° to 38.0° C. instead of 30° C. as recommended by Kendall. Our procedure was as follows: Sheep brain free from blood and membrane was ground finely in a meat chopper. The material was extracted once with two volumes of 95 per cent ethyl alcohol\* and the dry tissue residue was next extracted in the same manner twice with two volumes of U.S.P. benzol. The

\*Twelve to sixteen hours at 20-22° C.

resulting finely powdered dry material added in the proportion of two grams to 10 cc of 0.85 per cent sodium chloride solution, was mixed thoroughly and sterilized in the autoclave at 15 pounds' pressure for twenty minutes. In our hands this medium appeared to offer no special advantage in carrying on the culture transplants. The complicated technique of preparation also discouraged the use of this medium. Whether or not this substrate is essential for the successful cultivation of the virus in conjunction with the VB medium remains to be determined.

Essentially the procedure was to inoculate the virus material into the EB medium and to make subplants at suitable intervals to the VB substrate in which the cultures were main tained by serial dilution. In a number of instances successful growth was obtained by means of the VB medium alone. Undoubtedly the two mediums exerted different effects upon growth, morphology, and virulence. Reversible morphologic changes were unusually striking when observed over long periods of time.

Tubes found to contain ordinary bacteria that might be contaminants were discarded. Generally these could be detected after twenty-four to forty-eight hours of incubation. Cultures that gave macroscopic evidence of growth only after fourteen to twenty-one days were set aside for study and subplanting. At such times a definite faint turbidity changing to an unmistakable clouding of the culture medium took place in the deeper levels of the supernatant layer of fluid in the tubes containing VB medium. The brain tissue immediately beneath this fluid was now covered with a fine grayish white layer of material. The control tubes remained entirely clear during this period and ruled out the possibility of tissue autolysis causing the turbidity observed in the inoculated tubes (Fig. 1). A sterile platinum loop applied to this layer of material brought away a slightly viscous fluid adhering momentarily by a fine thread as the loop was withdrawn.

Film preparations were allowed to dry on slides *without heat fixation* and were stained by the methods of Wright, Gram, and Giemsa. It is noteworthy that the first generation cultures of filtrates from several strains of poliomyelitis virus yielded a definite turbidity in the EB culture medium without revealing organisms in numerous film preparations. Subsequent transplants to the VB medium or to corresponding EB medium, however, were found to contain living organisms. These were absent in the control culture tubes inoculated with material from normal monkey brain and the viruses of herpes, encephalitis, and neurovaccinia. Subplants from apparently negative EB medium tubes showed growth microscopically in the VB medium as early as ten to fourteen days before this could be demonstrated in the original culture tubes (Table I).

TABLE I

SUMMARY OF CULTURE SUBPLANTS OF POLIOMYELITIS VIRUS (FIRST THREE GENERATIONS)

DATE OF INOCULATION	GENERATION	CULTURE MEDIUM	DATE OF POSITIVE GROWTH
8/19/31	1	EB	9/14/31 (Negative 9/10/31)
8/28/31	2	VB	9/4/31
8/28/31	2	VB	9/10/31
8/28/31	2	EB	9/14/31
8/28/31	2	EB	9/14/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31

The organisms from subplants could be grown under strict microbiosis on the surface of a solid culture medium prepared by adding eight parts of VB medium to ten parts of 2 per cent veal infusion agar, containing 0.4 per cent  $\text{NaH}_2\text{PO}_4$ , having a  $\text{pH}$  of 7.6. This medium in Petri plates, or slanted in test tubes, yielded moderate surface growth after one week or ten days.



After two or more weeks of incubation the growth became more profuse. Successful secondary cultivation was accomplished in this manner by the use of the Olitsky-Boez apparatus<sup>32</sup> equipped with a palladiumized asbestos catalyst.

Subplants from positive cultures were made on aerobic blood agar plates and ordinary agar slants and in brain heart infusion (Bacto) incubated aerobically and anaerobically under sterile liquid albumin. No growth could be detected microscopically or microscopically after one month.

*To summarize*—Cultivation experiments were attempted with Berkefeld N filtrates of 5 per cent suspensions of brain and spinal cord from monkeys inoculated with poliomyelitic virus and developing typical experimental poliomyelitis. Seven strains of virus (all representing the original Flexner virus after propagation in series of monkeys) were studied in a special medium described here as EB and VB and containing minced sheep brain. Positive cultures were obtained under anaerobic conditions from such virus filtrates and subplants from all positive tubes have yielded successful growth in series. Up to the present time these cultures are in the nineteenth subplant and the organisms in the original tubes have remained viable to date after an incubation period of more than twenty-two weeks at 37.5° to 38.0° C.

Successful cultivation on solid medium has been accomplished under strictly of subplants anaerobic conditions by means of the Olitsky-Boez apparatus. The culture medium for this purpose was prepared by addition of VB sheep brain medium to buffered veal infusion agar, free from salt and peptone, and adjusted to pH 7.6.

#### MORPHOLOGY

In film preparations made from inoculated culture medium, and stained by the method of Wright, the organisms were clearly defined notwithstanding the presence of microscopic bits of tissue, cellular detritus, and occasionally variable amounts of granular deposit. The Giemsa stain was found unsuitable owing to the lack of contrast in the field and a tendency toward overstaining. With the Gram technique the organisms could not be demonstrated in the early subplants although they were present in duplicate specimens prepared with the Wright stain. In older cultures and later subplants the bodies could be seen with the Gram stain and were weakly gram-positive. Hematoxylin-eosin was also found satisfactory. Under a magnification of 1500 to 2000 diameters the minute organisms with Wright stain were seen as blue or violet ovoid bodies occurring singly, in pairs, or irregular clusters, very rarely in short chains, and in densely packed masses. They varied in size from the lowest limit of visibility to 0.05 or 0.1 to 0.2 micron. Enormous numbers were seen in particles of brain tissue for which the organisms exhibited a marked predilection. They appeared on closer inspection to have a sheath-like surrounding envelop that favored the formation of masses or clumps (Figs. 2, 4).

In the more remote subplants the minute bodies were larger and infrequently associated with extremely large globular or coccoid forms ranging in size from 2 to 3 or more microns in diameter. These were usually pink or reddish in color and unevenly stained, sometimes resembling the low power picture of polymorphonuclear leucocytes. Among these were also found vacuolated struc-

<sup>32</sup> -34 C has proved more satisfactory

tures having the appearance of an empty shell, and reminiscent of the "Pettenkotelnatoimen" described by Kuhn.<sup>46</sup> The large bodies were seen frequently in the first few generations derived from the primary culture, particularly when subplants were made into EB medium. Frequently, also, these forms appeared in cultures in which the small organisms had undergone disintegration and the culture appeared to be growing poorly. The minute bodies and small forms in gen-

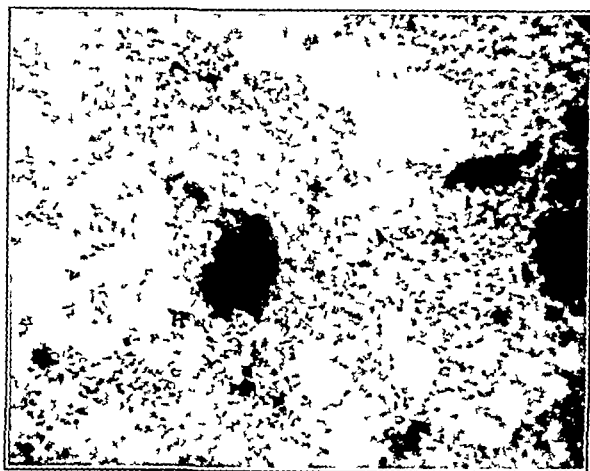


Fig. 2—Film preparation virus culture fifth subplant ten days growth showing organisms in VB brain tissue medium. The dark are is represent dead nuclear material in the nerve tissue (Wright stain  $\times 1500$ )

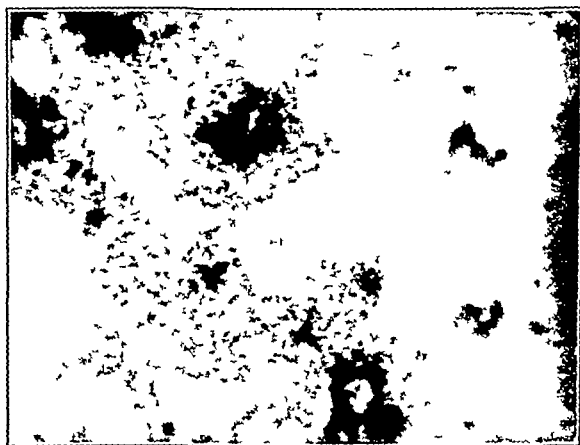


Fig. 3—Film preparation of virus culture showing minute bodies and masses in relation to nuclear structures in the brain tissue (Wright stain  $\times 1500$ ) (VB medium)

eral predominated in VB culture medium, and in the later generations as the large organisms became rare the small bodies appeared in virtually pure culture. However, a subplant from tubes containing one type exclusively might yield the other or a mixture of the two (Figs 5-7). In a number of subplants the minute ovoid bodies appeared elongated and the surrounding sheath-like structure gave the single or grouped organisms the appearance of fusiform bacteria.

*Morphology in Culture Filtrates*—Berkefeld N filtrates from cultures con-

taining the typical small and associated larger forms yielded positive cultures in VB medium after eight to thirty days' incubation. Film preparations revealed clusters of minute organisms and slightly elongated sheath-like forms stained blue. Large forms were not found. Filtrates from cultures containing only the larger globular bodies (as observed in several film preparations) were negative for growth after four weeks of incubation. Typical small forms developed in the fil-

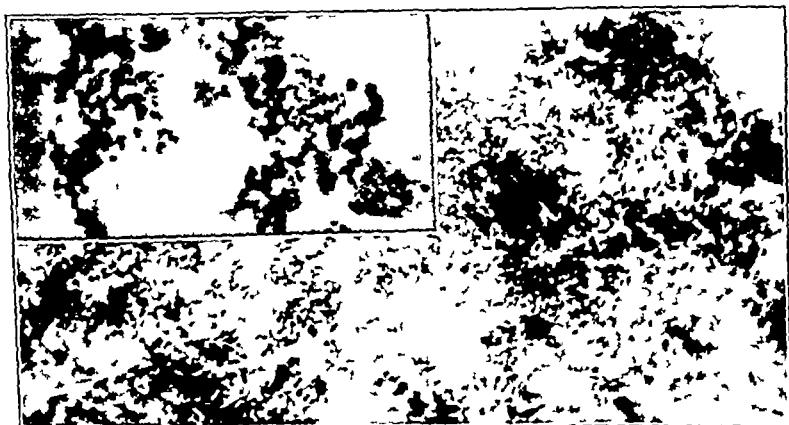


Fig. 4.—Film preparation of virus culture showing zooglyphic masses and sheath-like structure of bodies. A few large bodies are seen in the field. (Wright stain  $\times 1500$ ) (LB medium)

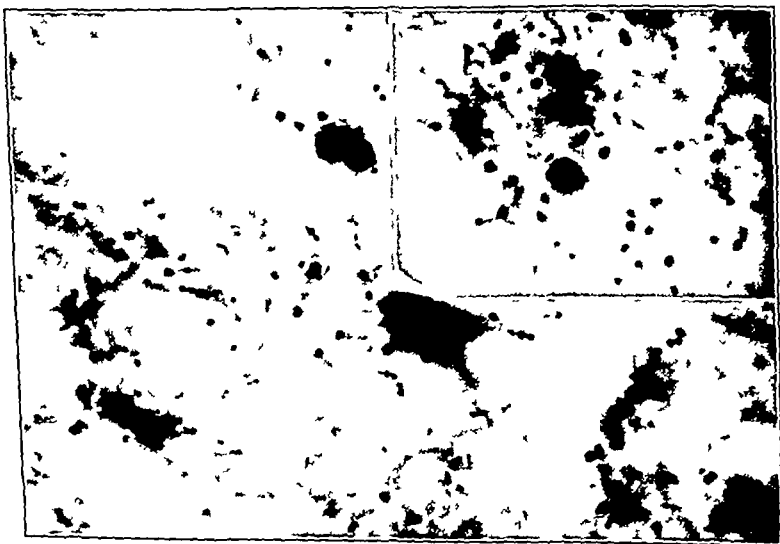


Fig. 5.—Film preparation of virus culture with subplant showing relative size of minute bodies and large globular forms in comparison with nuclear structure in brain tissue. (Wright stain  $\times 1500$ )

trates in six trials with cultures that originally contained only the typical minute bodies.

*Comment*—From these preliminary filtration experiments certain permissible deductions might be made. These appear to bear some relation to the microscopic appearance observed in film preparations of cultures taken at different times. A given culture contains organisms that are optically invisible and these

develop into visible forms. For the moment the various stages of growth and possible life cycle may be disregarded. It is important to note that the mere recultivation of identical organisms from a Berkefeld filtrate of a culture need not imply the actual passage of the original bodies, however small, through the filter. A rea-

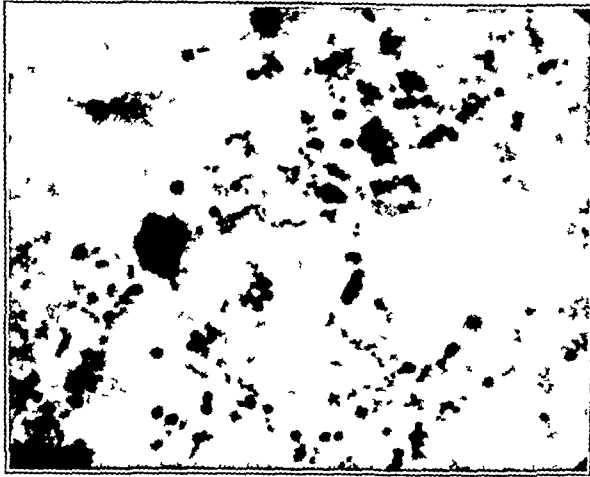


Fig. 6—Film preparation of virus culture sixth subplant showing preponderance of large forms developing in the brain tissue (Wright stain  $\times 1500$ )

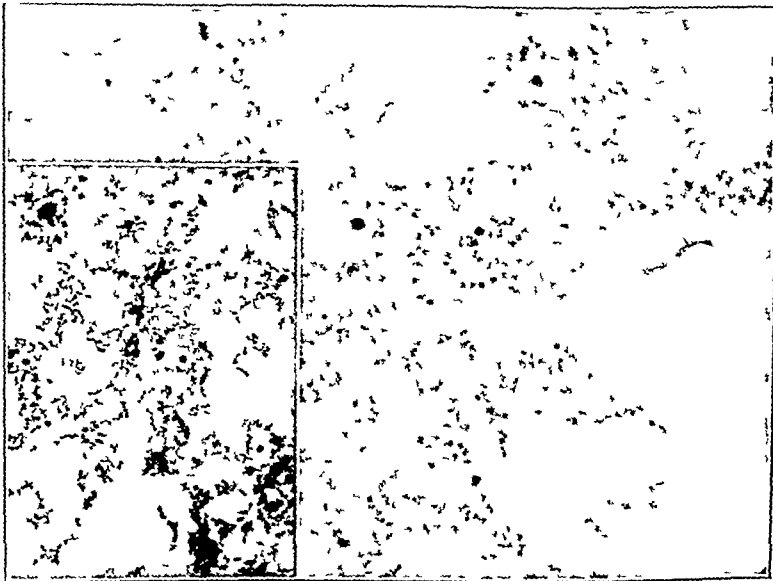


Fig. 7—Film preparation of virus culture sixth subplant showing proliferation of organisms in the matrix of brain tissue (Wright stain  $\times 1500$ )

sonable interpretation of the experiment suggests that ultramicroscopic invisible forms that are present in the filtrate may develop later into visible bodies in the culture medium. This inference cannot be accepted as a definite hypothesis without more elaboration of these experiments. In the few instances studied, no microorganisms could be demonstrated in stained film preparations made at various

times from Berkefeld N filtrates of cultures. These filtrates were subjected to prolonged centrifugalization at the highest possible speed and the stained material from the tip of a Hopkins centrifuge tube was examined microscopically under a magnification of 2000 to 3000 diameters.

*To Summarize*—The typical organism isolated from Berkefeld N filtrates of poliomyelitic brain and spinal cord tissue was a very minute dot-like or ovoid body ranging in size between 0.05 to 0.1 or 0.2 micron. It appeared to have a sheath-like structure tending to cause the bodies to adhere in clumps or irregular masses. At times they occurred in small clusters, or singly or in pairs and rarely in very short chains. With the Wright stain the organisms stained blue or deep violet, sometimes reddish violet depending upon the  $P_H$  of the culture medium at the time. The reaction to Gram's stain was weakly positive in older cultures and in late generations. In primary culture and in the early subplants the bodies could not be demonstrated by the Gram method. They were clearly seen with a magnification of 1500 to 2000 diameters and appeared to have multiplied abundantly in the bits of brain tissue that constituted the culture medium. After several weeks of incubation at 37.5° to 38° C. the organisms became somewhat enlarged and stained irregularly. These cultures containing the small forms were "filterable" through Berkefeld N filters just as were the original typical minute bodies. Filtration experiments with the cultures and the recovery of the original organisms from the Berkefeld filtrate suggested the passage of temporarily invisible forms capable of developing later into visible ones in the culture medium. The actual passage of the original minute organism through a filter was not implied. The possibility of harmless "contaminants" being present in the company of typical bodies was also recognized. These associated organisms may exhibit remarkable pleomorphism and bizarre stages of growth. The deduction may not be made without further elaboration of the study that the different forms seen in any film preparation are necessarily related to a life cycle. Such an hypothesis, however tempting, might be hazardous at this time.

#### INFECTIVE POWER OF CULTURES IN MONKEYS

In order to establish the possibility of causal relationship of the organism to poliomyelitis, attempts were made to induce experimental infection in monkeys. For this purpose cultures were used that had been isolated from Berkefeld N filtrates of brain and cord of a monkey experimentally infected with a known virus. This strain of virus, injected intracerebrally in 0.4 to 0.5 c.c. amounts of a Berkefeld N filtrate of a 5 per cent suspension of glycerolated brain and cord produced typical poliomyelitis in seven to eight days. Smaller doses were infective only rarely or not at all in numerous attempts to arrive at an approximate minimal dose in medium sized monkeys that were at our disposal. The cultures used in the experiments to be described were obtained from a monkey with the following history.

*Experiment 1*—*Macacus rhesus* inoculated intracerebrally on February 28, 1931, with 0.5 c.c. of a Berkefeld N filtrate of a 5 per cent suspension of glycerolated virus recently obtained from a monkey in the paralytic stage of poliomyelitis (courtesy of Dr. Simon Flexner, Rockefeller Institute). March 6, excitement and lessened activity were observed. March 7, body tremor, facial paralysis, and paralysis of right arm were noted. March 9, ataxia, weakness of both legs and left arm were observed. The animal was prostrate and unable to move its legs and arms later in the day. Etherized. The lesions in gross and histopathologic sections were

characteristic of experimental poliomyelitis. Infected nervous tissues of this animal were preserved in sterile 50 per cent glycerol and stored in the ice chest at 4° C for five and one half months. At this time Berkefeld N filtrates of a 5 per cent suspension of the brain and cord were inoculated into the special culture medium from which were derived the cultures that have been described.

Monkeys were inoculated with materials from the cultures in their third, fourth, seventh, eighth, tenth, eleventh, and thirteenth subplants. It should be noted that the fourth transfer already represented a dilution of about  $2 \times 10^{-3}$  of the original inoculum that had been used for primary cultivation in amounts of 0.1 c.c. of a Berkefeld N filtrate. This amount of virus by itself was considerably below the known infective dose aside from its serial dilution in the subplants.

#### METHOD OF PREPARATION AND INOCULATION OF CULTURE MATERIAL

The material used for inoculation of monkeys consisted of the supernatant contents of several culture tubes incubated eight to twelve days, well shaken and mixed when removed from the incubator and centrifuged for two to three minutes at 125-150 R.P.M. In a few instances four, six, and twenty-five day old cultures were used. As a general rule subplants were prepared from tubes that had been incubated for two to three weeks, occasionally from some six to seven weeks old, and in two instances from sixty-five and one hundred day old cultures. Experience showed that cultures less than ten days old were best suited for inducing an infection. In one series of experiments (eleventh subplant) sedimented organisms, washed 3 or 4 times with salt solution, were used. All materials were examined microscopically in film preparations before injection into monkeys and only satisfactory specimens free from ordinary contaminating bacteria were employed. The culture used in the inoculation experiments will be designated as Strain 3.

*Certain points that may modify the outcome of inoculations should be emphasized here. It is especially important to secure an actively growing culture preferably not more than nine days old. This can be ascertained from film preparations made at the stated time. The organism has certain peculiarities and fastidious requirements. It will be noted that multiplication takes place in the bits of brain tissue constituting the major part of the culture medium. Hence it is essential to secure material from cultures having abundant organisms in this dead yet chemically and biologically suitable tissue substrate. The amount of material used for inoculation is an important factor, notably in animals such as the monkey that is only relatively susceptible to poliomyelitis. To cause a primary infection in the monkey will require a considerable amount of culture material. This amount is not in any sense extraordinary considering the type of material inoculated and the slow growth of the organism. Once the disease has been induced, however, serial passage can be effected in the monkey by means of small amounts of Berkefeld filtrates or of suspensions prepared from the nervous tissues of the infected animals.*

The following method was adopted. The contents of a number of culture tubes, generally 4 to 6 in number, were transferred under strict aseptic precautions to centrifuge tubes having a capacity of 50 c.c. In order to facilitate the passage of tissue particles, a 10 c.c. pipette was used with the narrow outlet broken off to increase the diameter. Suction was applied by means of a large rubber bulb at-

tached to the mouthpiece. After light centrifugation at the speed indicated, all the material overlying the gross sediment was carefully removed. This material passed without difficulty through the 21 and 22 gauge needles used in the course of inoculations, and could be injected safely into the blood stream either by way of a suitable vein or more expeditiously by direct injection into the heart.

*Route of Inoculations*—Several different routes through which infection might be accomplished were tried. The primary object was to induce localization of the organism in the brain without the theoretical possibility of virus "contamination." In order to favor penetration of the virus culture material, certain accessory procedures were used. The method of choice was to bring the organisms less directly and more slowly to the brain by way of the blood stream. To this end an injection of 10 per cent starch solution or physiologic salt solution was given into the brain and the culture into the blood stream and peritoneal cavity. In a few instances serial passages were aided by a preliminary injection into the spinal canal of normal monkey serum or of salt solution. When direct inoculation of organisms into the brain was attempted, this method was always combined with the intravenous and intraperitoneal injections or with the intravenous alone. The inoculation into the cerebrum of cultures, starch solution or salt solution served as an irritant to the brain tissues. Although we have had successful results with a single injection of a small amount of culture into the brain alone, it is again stressed here that virulence and other unknown factors may modify the end-results.

Flexner<sup>27</sup> has emphasized the fact that the negative infecting power of massive intravenous doses of a virus can be altered through an aseptic meningeal irritation induced by the intraspinal or intracerebral injection of a normal serum or of salt solution. Similarly, it has been shown for Borna virus<sup>28</sup> that the infection can be produced experimentally according to the same principle. Sawyer and Lloyd<sup>29</sup> studying yellow fever virus were able to infect mice by the intraperitoneal route following intracerebral injection of a 2 per cent solution of starch.

In a preliminary experiment direct inoculation was made into the cerebral hemisphere of two monkeys and into the peritoneal cavity of a third animal following an intracerebral injection of physiologic salt solution. The cultures used were in the third and fourth subplants and these transfers represented a dilution of the original material cultivated of approximately  $2 \times 10^{-7}$  and  $2 \times 10^{-9}$ , respectively. Relatively small amounts of culture were injected in order to test the virulence of the organism. Apart from a variable degree of weakness of certain groups of muscles and clinical symptoms suggestive of experimental poliomyelitis, no typical paralyses were observed in this first series.

*Experiment 2—Macacus rhesus 17* September 5, 1931, 1 c.c. of supernatant material from one positive culture Strain 3, incubated four days in the third subplant in VB medium, was inoculated into the cerebral hemisphere. The animal remained normal until September 13, on the eighth day following inoculation, when tremors of the head and body, slowness in movement, weakness of extremities, loss of appetite, and fatigability were noted. This condition improved gradually during the succeeding three days. September 17, appeared normal.

*Macacus rhesus 27* September 11, 1931, 1 c.c. of supernatant material from Strain 3 incubated nine days in the third subplant in VB medium was inoculated into the left cerebral hemisphere. September 30, no symptoms having developed at this time, a second inoculation was made into the hemisphere with 1 c.c. of a culture incubated twenty one days, in the fourth

subplant The animal remained normal until October 6, when it became quiet and could not chew its food There was a suggestive right facial paralysis and weakness of the buccinator muscles on the same side October 7, right facial paralysis was more advanced, food dropped from mouth and the animal was unable to empty pouch, weakness of arms and legs and back Improvement occurred during the next four days October 12, appeared normal

*Macacus rhesus 16* October 1, 1931, 3 c.c. physiologic salt solution were injected into the left cerebral hemisphere and 6 c.c. of a culture incubated twenty five days in the fourth subplant were inoculated into the peritoneal cavity October 21, quiet, slow in movement, loss of appetite October 22, weakness of deltoid muscles and extremities, especially on right side, body tremor, and tremor of arms and legs were observed Gradual improvement until October 25, when animal appeared quite normal

In the next series of experiments somewhat larger amounts of culture were inoculated These were by no means massive doses but represented pooled material from three to six culture tubes It will be recalled that the enormous numbers of "globoid bodies" inoculated by Olitsky represented material from "cultures" contained in thirty or more tubes

*Experiment 3—Macacus rhesus A* October 1, 1931, pooled supernatant material from 3 positive cultures incubated twenty five days in the fourth transplant was prepared from Strain 3 Two c.c. physiologic salt solution were injected into the right cerebral hemisphere, and 6 c.c. of the culture were injected into the peritoneal cavity October 23, 0.5 c.c. culture was injected into the left hemisphere and 1 c.c. into the peritoneal cavity October 26, head and body tremor, ataxic gait, general weakness, completely paralyzed and prostrate later in the day Died

*Autopsy* Meninges were slightly congested A small area of softening was disclosed near the site of the first inoculation No hemorrhage was noted The cord in the area of the cervical and dorsal enlargements showed injection and petechial hemorrhage in the region of the anterior horn cells Smears from these areas and ordinary culture tests from the brain and cord were negative Microscopic Examination In the forebrain only a slight mononuclear cellular reaction was observed Proliferation of the glial cells was very slight to moderate in extent Nerve cell degeneration was not pronounced The medulla showed some round cell infiltration and moderate perivascular infiltration Degeneration of nerve cells was not marked In the cord, particularly the cervical and dorsal enlargements, were found moderate lymphocytic and leucocytic infiltrations, and slight destruction of the anterior horn cells Neurophagocytosis was not very definite

A 5 per cent saline Berkefeld N filtrate was prepared from pieces of the brain and cord and 1 c.c. of this material was injected intracerebrally into a monkey (No 43) This animal developed typical experimental poliomyelitis on the sixth day with complete flaccid paralysis on the seventh day (Fig 8)

Cultivation experiments in VB medium were carried out with Berkefeld N filtrate prepared from 10 per cent suspensions of the brain and cord of Monkey A These tissues had been preserved in 50 per cent glycerol for ten weeks The typical small organisms were found in the tubes of culture medium after an incubation period of eighteen to twenty-one days

*Experiment 4—Macacus rhesus B* October 21, 1931, 2 c.c. of a 10 per cent starch solution were injected into the hemisphere and a culture prepared from Strain 3 in the fourth subplant incubated six days was inoculated as follows 6 c.c. were injected into the peritoneal cavity and 8 c.c. intravenously This subplant was derived from a culture in the third generation that had been kept at incubator temperature forty two days October 31, well marked head and body tremor, excitability, staccato cry November 1, paralysis of extremities on right side, left arm and left leg very weak, ataxic gait, fur ruffled, high excitability (Fig 9) November 2, complete paralysis, prostrate, moribund Etherized

*Autopsy* Meninges and superficial cortical vessels were moderately injected On cut



surface the brain and cord showed edema and areas of petechial hemorrhage in the gray matter and particularly in the region of the anterior horn cells. These changes were more marked in the cervical and dorsal enlargements of the spinal cord. Ordinary cultures and film preparations from the surfaces of the fresh nervous tissue were negative for microorganisms.

Sections from the cerebellar cortex showed slight venous congestion in the pia. The arachnoidal endothelium appeared normal. There was a small hemorrhage about which were a few lymphocytes, and scattered sparsely in the pia were mononuclear cells, mostly of undifferentiated type, occasionally showing the morphology of plasma cells. The undifferentiated type of cell possessed a relatively smaller and more deeply staining nucleus than the plasma

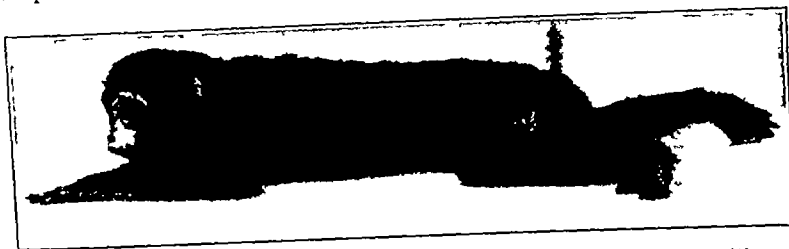


Fig. 8—Monkey 13. Complete flaccid paralysis seven days after inoculation with poliomyelitic nervous tissue from monkey A infected with culture Strain 3.

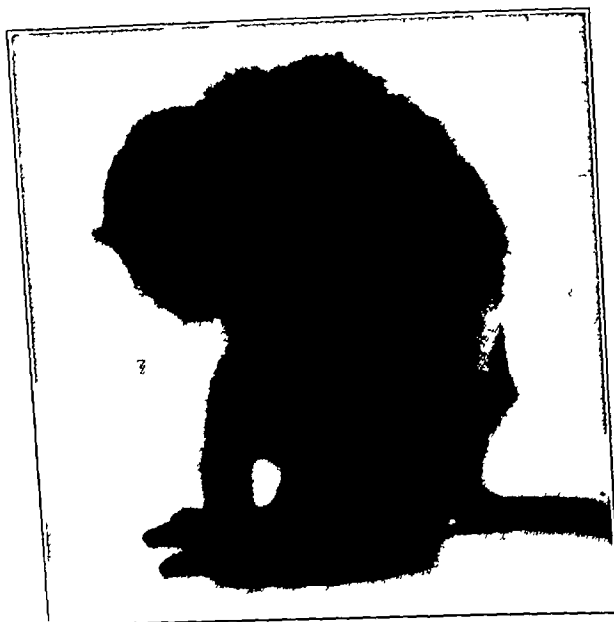


Fig. 9—Monkey 39. Complete paralysis upper extremities neck and back muscles and beginning paralysis of lower extremities. Tenth day after inoculation with culture in fourth subplant in VB medium six days growth.

cell, and the cytoplasm was frequently more abundant, less sharply outlined, and much less basophilic than the plasma cell. The cortex proper showed normal lamination. The nerve cells appeared negative. No inflammatory reaction or degenerative alteration was seen.

Sections from the basal ganglia showed a bit of ependymal lining to be normal. In the tissue generally, there were a few scattered venules showing distinct perivascular infiltration with lymphocytes and plasma cells.

Sections from the cerebellum showed a slight scattered infiltration with plasma cells and mononuclear cells. The cerebellar tissues, generally, appeared negative.

Sections from the spinal cord showed in the pia rather slight inflammatory reaction, except in the extension of the pia into the anterior fissure, where a variable amount of lymphocytic

and plasma cell infiltration occurred in the different sections. The general architecture of the anterior horns, and to a lesser extent posteriorly, was disturbed by diminution in number of the anterior horn cells. Some were absent, others represented by fragments or nerve cells, and some showed infiltration about them. Neurophagocytosis was quite prominent.

There were also tiny patches of cellular infiltration independent of obvious degenerating cells. A few petechial hemorrhages were seen. The cellular exudate about degenerating areas



Fig 10—*Monkey 39* Section spinal cord showing extensive destruction of anterior horn cells and perivascular infiltration, vascular hemorrhages and congestion and diffuse round cell infiltration, focal accumulations of leucocytes and moderate neurophagocytosis.

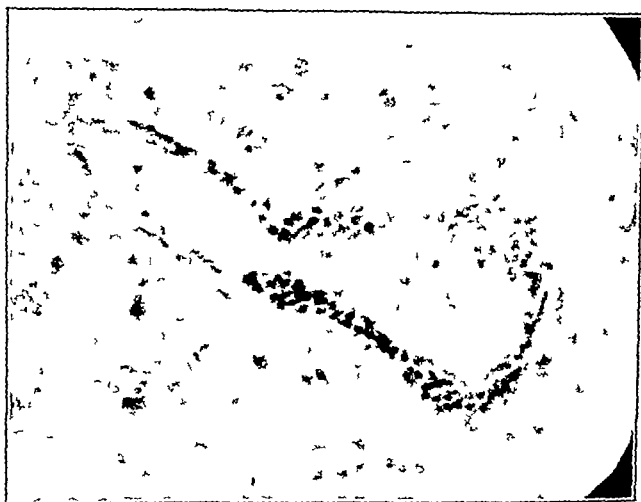


Fig 11—*Monkey 39* Section brain Nissl stain showing round cell perivascular infiltration and nerve cell necrosis (x400).

consisted of undifferentiated mononuclear cells, occasionally slight perivascular infiltration occurred, and there was apparently some beginning hypertrophy of the glial elements in this region (Figs 10-13).

The next experiment was an attempt to transmit experimental poliomyelitis to normal monkeys from those that had been infected by means of the culture.

Three monkeys were inoculated with suspensions of Berkefeld N filtrate prepared

pared from the brain and cord of Monkey B in Experiment 4. All three control animals developed typical poliomyelitis, two within eight days and one on the tenth day. These results are given in Experiment 5.

*Experiment 5 — Macacus rhesus 36* November 9, 1931, 2 cc normal monkey serum were injected intraspinally. November 10, 1 cc of a 10 per cent suspension prepared from the

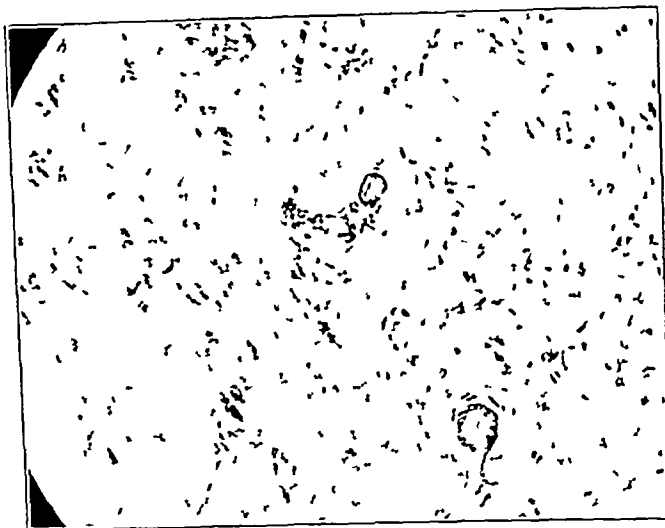


Fig. 12 — *Monkey 39*. Section brain Nissl stain showing round cell perivascular infiltration and nerve cell necrosis ( $\times 100$ )

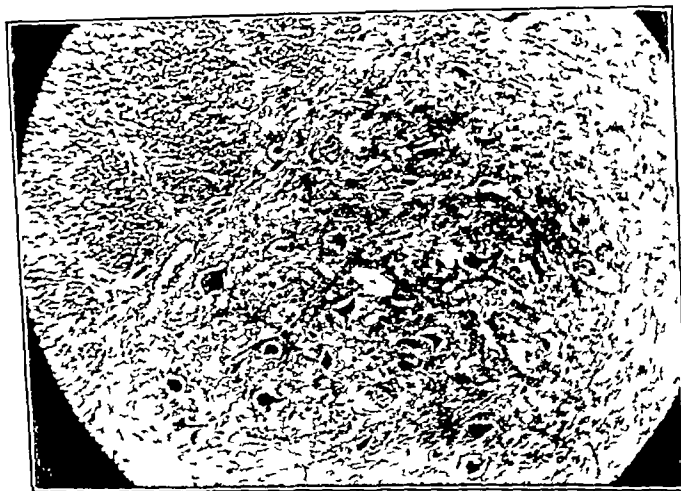


Fig. 13 — *Monkey 29*. Normal monkey. Section spinal cord. Note number normal intact nerve cells and absence of inflammatory changes in comparison with Fig. 10

brain and cord of Monkey B was inoculated into the right cerebral hemisphere. November 16, excitement, tires easily, fur ruffled. November 18, complete paralysis of the extremities and back muscles, prostrate. November 22, died. The gross and histopathologic examinations revealed characteristic pronounced lesions of experimental poliomyelitis (Figs. 14-16).

*Macacus rhesus 37* November 9, 1931, 1 cc of a 10 per cent suspension prepared from the brain and cord of Monkey B was inoculated into the cerebral hemisphere. November 16, weakness of extremities, ataxic gait, body tremor, excitability, head tremor, staccato cry, right facial paralysis. November 17, complete paralysis, prostrate, unable to move arms and legs.

Etherized The gross and histopathologic examinations revealed characteristic well marked lesions of experimental poliomyelitis

*Macacus rhesus* 38 November 9, 1931, 1 cc of a 10 per cent saline Berkefeld N filtrate prepared from the brain and cord of Monkey B was inoculated into the cerebral hemisphere November 19, head and body tremor, excitability, weakness of extremities and back muscles, ataxia November 20, complete paralysis of extremities, prostrate, unable to move arms and

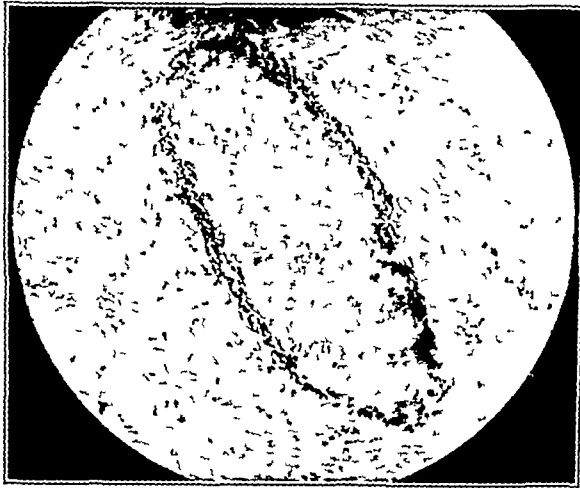


Fig 14—*Monkey 36* Complete paralysis eighth day died twelfth day inoculated with nervous tissue from monkey 39 representing second virus culture passage Section of brain showing perivascular infiltration of cerebral blood vessel and diffuse round cell infiltration

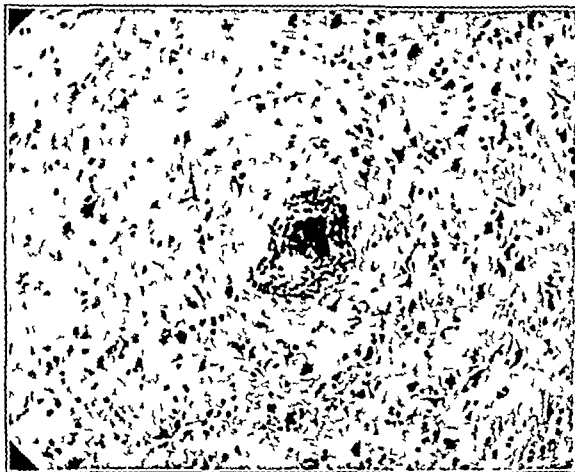


Fig 15—*Monkey 36* Section of brain showing round cell infiltration of smaller blood vessel and diffuse inflammatory reaction

legs Etherized Gross and histopathologic examinations revealed typical and pronounced lesions of experimental poliomyelitis (Fig 17)

Cultivation experiments in VB medium were made with 10 per cent saline Berkefeld N filtrates and suspensions prepared from the brain and cord of Monkey B and of Monkeys 37 and 38 In the first and successive transfers, minute bodies resembling the typical original cultures, were isolated The nervous tissues from these animals had been preserved in 50 per cent glycerol and stored in

the ice chest at 4° C for periods ranging from three hours to four days, and up to and including seven weeks. Tests for sterility in ordinary culture medium were uniformly negative after three to four weeks of incubation.

Two normal monkeys were inoculated with one of these cultures recultivated from the brain and cord of Monkey 38. One animal developed symptoms and

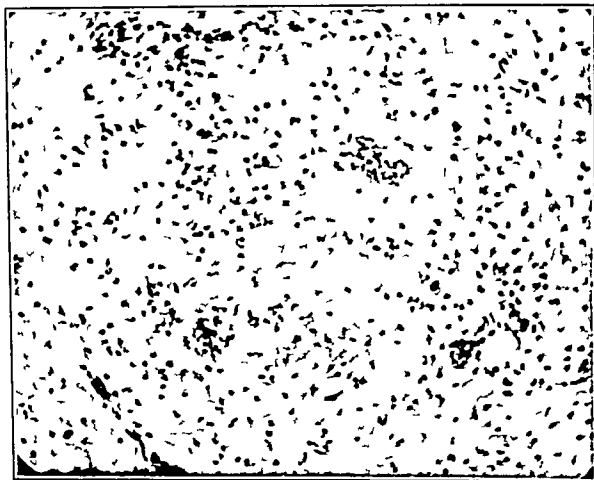


Fig 16—*Monkey 36* Section of spinal cord showing focal accumulation of round cells with marked destruction of nerve cells diffuse round cell infiltration and neurophagocytosis

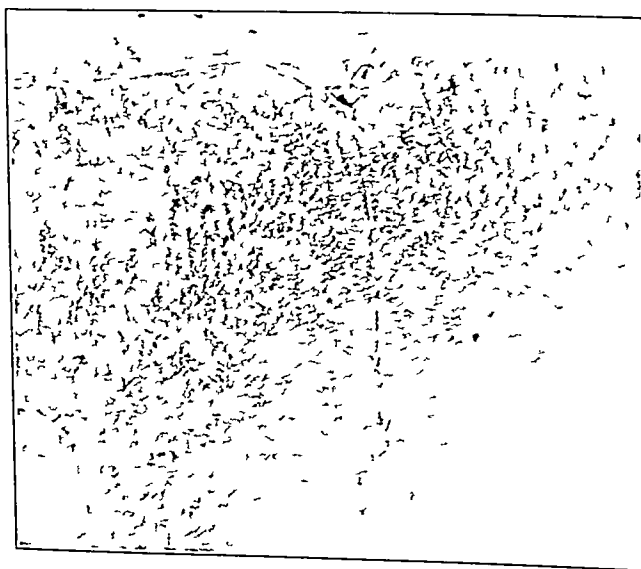


Fig 17—*Monkey 38* Complete paralysis eleventh day inoculated with Berkfeld filtrate nervous tissue from monkey 29 Section of spinal cord showing vascular lesion extending from meninges inward along anterior fissure diffuse infiltration of blood vessel

paralysis on the twelfth day following inoculation with complete recovery. The second animal showed symptoms on the fifteenth day, became prostrate and died on the sixteenth day following inoculation. The culture used was prepared from a pooled seven day growth of organisms in V B medium and representing the second subplant. The results are summarized in the following experiment

*Experiment 6—Macacus rhesus 59* December 12, 1931, 2 c.c. of a 10 per cent starch solution were injected into the right cerebral hemisphere, 5 c.c. of pooled culture material intravenously, and 5 c.c. into the peritoneal cavity. December 19, animal was normal. December 24, tires easily, left deltoid muscle weak. December 28, excitability marked, left deltoid defi-



Fig 18—*Monkey 53* Complete paralysis eighth day, died on ninth day. Inoculated with suspension nervous tissue from monkey 38. Section spinal cord showing diffuse infiltration, focal accumulation of round cells and vascular lesion extending inward from meninges. (Third virus culture passage.)

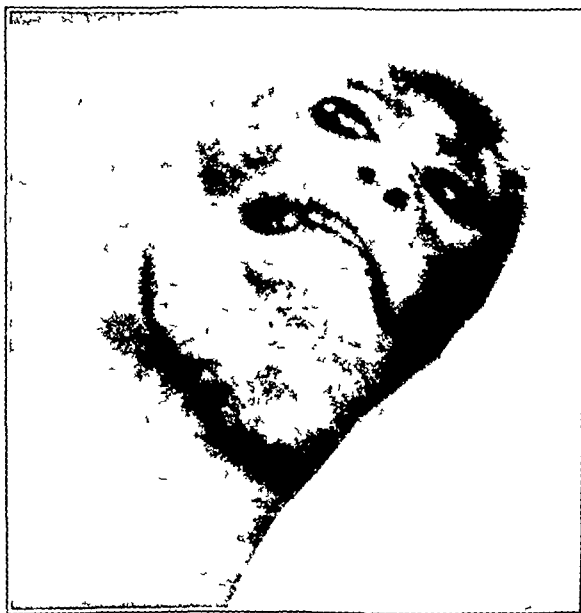


Fig 19—*Monkey 56* Complete paralysis bulbo spinal infection and death on fourth day. Inoculated with Berkefeld filtrate nervous tissue from monkey 38. Left facial paralysis on fourth day after inoculation. (Third virus culture passage.)

nitely paralyzed. No other involvement was observed. January 4, 1932, excitability decreased, left deltoid still weak. January 22, animal appeared to be quite normal.

*Macacus rhesus 61* December 11, 1931, 2 c.c. normal monkey serum were injected intraspinally. December 12, 2 c.c. culture were injected into the right cerebral hemisphere, 5 c.c.

intravenously and 5 cc into the peritoneal cavity. December 24, animal was normal. December 27, marked excitability. December 28, arms and legs very weak, weakness of back and neck muscles. Later in the day the animal became completely paralyzed and prostrate. It was unable to move arms or legs. Death from respiratory failure. The gross and histopathologic examinations revealed characteristic lesions of experimental poliomyelitis in the spinal cord and brain.

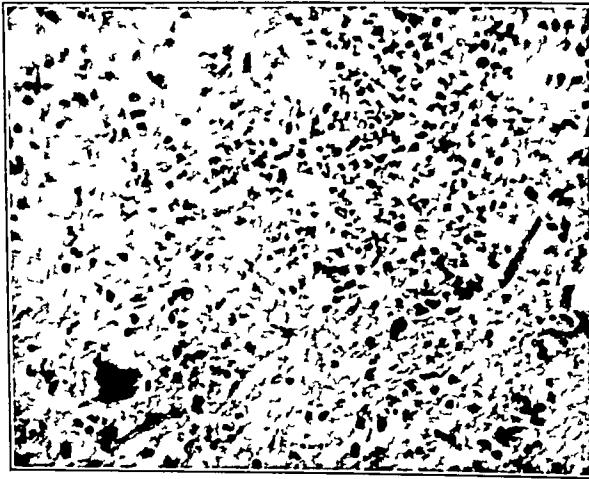


Fig 20—*Monkey 56* Section from spinal cord showing large focal accumulation of round cells and leucocytes and absence of nerve cells



Fig 21—*Monkey 56* Section from spinal cord, cervical enlargement showing pronounced neurophagocytosis, diffuse round cell infiltration and destruction of anterior horn cells

From these experiments it may be deduced that experimental poliomyelitis is transmissible with a culture recovered from culture-infected monkeys.

In the following experiment an attempt was made to induce experimental poliomyelitis in normal monkeys with materials prepared from the nervous tissues of animals representing a second successful passage of the culture Strain 3.

Four monkeys were inoculated with suspensions or Berkefeld N filtrates of materials prepared from the brain and cord of Monkeys 37 and 38 in Experiment 5. The results are summarized in Experiment 7.

*Experiment 7—Macacus rhesus 52* November 18, 1931, 1 c.c. of a 10 per cent saline Berkefeld N filtrate prepared from the brain and cord (one day in 50 per cent glycerol at 4° C) of Monkey 37 was inoculated into the right cerebral hemisphere. November 26, excitability, ataxia, complete paralysis, prostrate, unable to move arms and legs.

*Macacus rhesus 53* November 18, 1931, 1 c.c. of a 10 per cent suspension prepared from the brain and cord of Monkey 37 was inoculated into the right cerebral hemisphere. November 24, excitability, ruffled fur, staccato cry, tires easily. November 25 head and body tremor, left facial paralysis, left arm paralyzed. November 26, prostrate, unable to move arms and legs. November 27, died. The gross and histopathologic examinations revealed well marked lesions characteristic of experimental poliomyelitis (Fig. 18).

*Macacus rhesus 55* November 20, 1931, 1 c.c. of a 10 per cent suspension prepared from the brain and cord (three hours in 50 per cent glycerol at 4° C) of Monkey 38 was inoculated into the right cerebral hemisphere. November 24, excitability, ruffled fur. November 25, left facial paralysis, left arm paralyzed, head and body tremor, right arm and legs and back muscles very weak. November 26, complete paralysis, prostrate. December 2, died. His topathologic examination revealed typical lesions of experimental poliomyelitis.

*Macacus rhesus 56* November 20, 1931, 1 c.c. of a 10 per cent saline Berkefeld N filtrate prepared from the brain and cord of Monkey 38 was inoculated into the right cerebral hemisphere. November 24, head and body tremor, left facial paralysis, beginning paralysis of upper extremities, marked general weakness. Nine hours later, marked ataxia, more advanced paralysis of arms, and weakness of muscles of neck and back (Fig. 19). Later in evening, total paralysis, picture of rapidly progressing bulbar type of poliomyelitis, respiratory failure and death. Gross and histopathologic examinations revealed profound tissue changes characteristic of experimental poliomyelitis (Figs. 20 and 21).

Cultivation experiments in VB medium were made with Berkefeld N filtrates and suspensions prepared from the brain and cord of Monkeys 53 and 56. An organism resembling the original Strain 3 was isolated and grown in serial subplants.

From the preceding experiments one observes that the inoculation of suspensions or Berkefeld filtrates prepared from nervous tissue taken from monkeys experimentally infected with poliomyelitis by means of cultures induces typical clinical and pathologic effects. These effects are transmissible in series and, as shown in Experiment 7, appear to be augmented as a result of successive passage of the culture material through a series of monkeys. Notable in this respect is the definite shortening of the period of incubation. The recaptured "virus" appears to retain its original properties of filterability and pathogenicity. The results of some of the earlier experiments in monkeys and the transmission of poliomyelitis by passage through a series of animals are summarized in Table II.

The infective power of a culture in the eighth subplant was studied in the following experiment. The material represented a dilution of the original inoculum cultivated of approximately  $2 \times 10^{-1}$ .

*Experiment 8—Macacus rhesus 2636* November 25, 1931, the supernatant fluid from one culture was ground in a mortar with sterile quartz sand, and centrifuged for three minutes at 150 r.p.m. One cubic centimeter of the resultant turbid supernatant material was injected into the cerebral hemisphere. December 2, excitement, tremor and ataxia were noted, both arms almost totally paralyzed, prostrate. General weakness progresses and the animal was etherized.

*Autopsy*—Visible lesions were seen in the cervical and lumbar cord, viscera were normal. Smears from the brain surface and base and the site of inoculation were negative. Cultures in ordinary solid and fluid media were negative. Histopathologic examination revealed typical lesions of experimental poliomyelitis in the cord and brain.



TABLE II

SUMMARY OF DATA LAUFMAN IN WHICH AN ORGANISM ISOLATED FROM POLIOVIRUS VIRUS PHILIP WAS ADAPTED TO A CULTURE MEDIUM CONSISTING OF MINCED SHEEP BRAIN TISSUE SUSPENDED IN TILLIONE FREE, GLUCOSE VIAL INFUSION

Polyomyelitis Virus Filtrate, First Culture (3)

C 2

C 3

C 4

(ice and iper) M rhesus 5 (prostate nineteenth day)

(ice susp 10% brain and cord) M rhesus 13 (complete paralysis seventh day)

Polyomyelitis Virus Filtrate Culture, Fourth Generation

(ice starch sol, even and iper culture) M rhesus 19 (prostate eleventh day, died twelfth day, culture recovered)

M rhesus 36 ice susp 10 % brain and cord glycerolated 7 days, prostate eighth day, died twelfth day	M rhesus 37 ice susp 10 % brain and cord glycerolated 6 days, prostate eighth day, culture recovered	M rhesus 38 ice Berkefeld filtrate susp 10 % brain and cord glycerolated 6 days, prostate eleventh day, culture recovered
M 52 ice Berkefeld filtrate susp 10 % brain and cord, complete paralysis and prostate eighth day	M 53 ice susp 10 % brain and cord, complete paralysis seventh day, prostate eighth day, died ninth day, culture recovered	M rhesus 55 ice susp 10 % brain and cord, complete paralysis and prostate fifth day, died thirty second day
		M rhesus 56 ice Berkefeld filtrate susp 10 % brain and cord, prostate and died fourth day, culture re covered

*Macacus rhesus 2586* December 3, 1931, 1 c.c. of a 10 per cent suspension of material prepared from the brain, cervical and lumbar cord of Monkey 2636 was injected into the cerebral hemisphere. December 10, slight tremor, ptosis and ataxia were observed. December 11, tremor and ataxia were more marked and both arms were totally paralyzed. December 12, animal was prostrate and unable to move arms or legs. Etherized. Autopsy revealed visible gross lesions in the spinal cord. The viscera were normal. Cultures prepared with material from the nervous tissues were negative for organisms in ordinary culture media. Histopathologic examination revealed lesions in the brain and cord typical of experimental poliomyelitis.

In the next experiment virus cultures in the eleventh subplant were inoculated into a series of monkeys. These transfers represented a dilution of the original material cultivated of approximately  $2 \times 10^{-7}$ . Four monkeys were inoculated and three developed the typical clinical syndrome and paralysis of experimental poliomyelitis within seven days and the fourth animal on the tenth day. Of these, two received sedimented cultures washed three times with normal physiologic salt solution. The supernatant pooled material from five culture tubes that had been previously mixed and centrifugalized lightly for two to three minutes was sedimented at high speed. The resultant sediment containing the organisms was washed three times after preceding centrifugations. This material represented a culture in the eleventh subplant incubated eight days at  $37.5^{\circ}$  to  $38^{\circ}$  C.

*Experiment 9—Macacus rhesus C* December 17, 1931, 2 c.c. of washed culture were injected into the left cerebral hemisphere, 5 c.c. intravenously, and 5 c.c. into the peritoneal cavity. December 23, both arms paralyzed, neck and back muscles weak. Ten hours later, the animal was totally paralyzed, prostrate and moribund. Etherized. The infection advanced in the manner characteristic of a bulbar type. Gross and histopathologic examinations revealed characteristic lesions of poliomyelitis in the spinal cord and brain.

*Macacus rhesus D* December 17, 1931, 2 c.c. of a 10 per cent starch solution were injected into the left hemisphere, 5 c.c. of washed culture intravenously, and 5 c.c. into the peritoneal cavity. December 22, excitability, weakness of arms, slight muscle tremor, tires easily, beginning right facial paralysis. December 23, marked right facial paralysis, total paralysis of extremities, neck and back, prostrate. Etherized. The gross and histopathologic findings were typical of poliomyelitis in the brain and spinal cord.

*Macacus rhesus E* December 17, 1931, 2 c.c. of a 10 per cent solution of starch into the left hemisphere, 5 c.c. of pooled culture into the peritoneal cavity, and 5 c.c. intravenously. December 23, irritability and muscle tremors were noted. December 24, right facial paralysis, marked head and body tremor, weakness of right arm and leg, paralysis of left arm and leg, weakness of back muscles, and ataxia. Prostrate six hours later.

*Macacus rhesus F* December 17, 1931, 2 c.c. pooled culture into the left cerebral hemisphere, 5 c.c. into the peritoneal cavity, and 5 c.c. intravenously. December 25, normal. December 27, excitability and weakness of right deltoid and both legs were noted. December 28, legs completely paralyzed, right arm paralyzed and left deltoid weak. December 30, prostrate, and unable to move arms or legs.

A 10 per cent suspension was prepared from portions of the brain and cord of Monkey C, and of this material 0.4 c.c. was injected intracerebrally into seven monkeys. All these animals developed typical symptoms and paralysis followed by complete prostration after a period of from six to ten days. Four animals died on the seventh to the eighth day following inoculation. Gross and histopathologic examinations revealed typical and well marked lesions in the spinal cord, medulla and brain, that were characteristic of experimental poliomyelitis.

Cultivation experiments in VB medium were carried out with Berkeleyfield filtrates prepared from cerebral nervous tissue from Monkey C. These tissues had been preserved in 50 per cent glycerol for 14 days. Typical cultures were ob-

tained after an incubation period of 22 days in the culture medium. In all respects the organisms were identical with the original strain with which Monkey C had been inoculated.

Two monkeys were inoculated with cultures in the thirteenth subplant representing a dilution of the original material cultivated at approximately  $2 \times 10^{-27}$ . Both animals developed typical symptoms and were paralyzed within one week.

*Experiment 10—Macacus rhesus 68* January 6, 1932, 1 c.c. culture from pooled supernatant material from three tubes of the thirteenth transfer was injected into the right cerebral hemisphere, 5 c.c. intravenously and 5 c.c. into the peritoneal cavity. January 12, prostrate, complete paralysis, moribund. Fthricized.

*Autopsy*—The site of inoculation showed nothing unusual and film preparations were negative. The cut surfaces of the spinal cord, particularly in the cervical region and the medulla, showed hemorrhagic pin point areas. Histopathologic examination of the medulla and spinal cord revealed marked perivascular infiltration, nerve cell degeneration and neurophagocytosis and focal accumulations of lymphocytes. Meningeal reaction was pronounced in the cervical and thoracic and, to a lesser degree, in the lumbar cord.

*Macacus rhesus 69* January 6, 1932, 1 c.c. of culture from pooled supernatant material from three tubes of the thirteenth transfer was injected into the cerebral hemisphere, 5 c.c. into the peritoneal cavity and 5 c.c. intravenously. January 12, both legs paralyzed, left deltoid weak. January 13, prostrate, February 21, died.

*Autopsy* Tuberculosis of lungs. Histopathologic examination revealed typical lesions in the medulla, cord, and to a slight extent, in the brain. The changes were regressive and not like those seen in the acute stages.

The infective power of cultures heated for one hour at  $65^{\circ}\text{C}$  was next studied in two monkeys. One received a preliminary injection of starch solution and another an intraspinal injection of normal monkey serum.

*Experiment 11—Macacus rhesus 48* November 18, 1931, 1 c.c. of normal monkey serum was injected intraspinally and on the following day 5 c.c. of a suspension prepared from 3 pooled cultures in the fourth subplant, incubated nine days and heated for one hour at  $65^{\circ}\text{C}$ , were injected intravenously.

*Macacus rhesus 49* November 19, 1931, 1 c.c. of a 10 per cent starch solution was injected into the right cerebral hemisphere and 5 c.c. of a suspension prepared from 3 pooled cultures in the fourth subplant, incubated nine days and heated for one hour at  $65^{\circ}\text{C}$ , were injected into the peritoneal cavity, and 5 c.c. of the same material intravenously.

The monkeys remained well and were discarded after one month's observation.

Materials from culture tubes in the first, second, third, and fourth subplants that contained no demonstrable organisms were also inoculated into two monkeys as illustrated in the following experiment.

*Experiment 12—Macacus rhesus 18* September 5, 1931, 1 c.c. of pooled material from four negative tubes containing no demonstrable organisms, of the first to fourth subplants of Strain 3, was inoculated into the left cerebral hemisphere.

*Macacus rhesus 19* September 16, 1931, 1 c.c. of pooled material from three negative tubes containing no demonstrable organisms, of the first and second subplants of Strain 3, was inoculated into the right cerebral hemisphere. October 1, 1931, 2 c.c. physiologic salt solution were injected into the left hemisphere and 7 c.c. of material prepared from the contents of four negative tubes containing no demonstrable organisms, of the first and second subplants, were inoculated into the peritoneal cavity.

The monkeys remained well and were discarded after one month's observation.

From the preceding experiments one may observe that the inoculation in monkeys of organisms obtained from cultures of poliomyelitic tissue in VB me-

dium caused symptoms and signs and pathologic changes in the nervous system characteristic of experimental poliomyelitis. Successful experiments were accomplished with culture subplants representing a dilution ranging from approximately  $2 \times 10^{-7}$  and  $2 \times 10^{-9}$  to  $2 \times 10^{-7}$  of the original material cultivated, namely, 0.1 c.c. of a 5 per cent saline Berkefeld filtrate of poliomyelitic nervous tissue. In parallel experiments in which cultures heated for one hour at  $65^{\circ}\text{C}$  were used, the inoculations in monkeys were without effect. Likewise, additional experiments with materials derived from culture tubes that contained no demonstrable organisms resulted negatively.

#### INFECTIVITY EXPERIMENTS IN RABBITS, GUINEA PIGS AND MICE

It is well known that ordinary streptococci, or bacteria, when injected directly into the brain or into the circulation of monkeys or smaller animals give rise to symptoms that are not characteristic of poliomyelitis. As a general rule the outcome of inoculations with streptococci is referable to the production of frank meningitis, purulent cerebral abscesses, or septicemia and death within forty-eight hours or less.

The work of Rosenow and associates<sup>40</sup> and others<sup>41</sup> has been thoroughly re-investigated, notably by Bull,<sup>42</sup> Smilhe,<sup>43</sup> Amoss and Eberson,<sup>44</sup> and Olitsky and Long.<sup>45</sup> Flexner<sup>46</sup> in his earliest work had already shown that the rabbit was resistant to poliomyelitic infection. There should be no question as to the complete absence of any relationship between the streptococcus described by Rosenow or others, and what is recognized as poliomyelitis in the experimental animal. An examination of such protocols, as are given in these publications, establishes forthwith the fact that in no instance was poliomyelitis induced with cultures of streptococci. The clinical picture of the disease was conspicuously absent. The short period of incubation and rapid course of infection, its outcome, and the gross pathology did not correspond in the remotest degree to poliomyelitis.

With these points in mind it was thought desirable to study in the rabbit, and smaller laboratory animals, the behavior of the organisms isolated by us. Rabbits were inoculated into the cerebral hemisphere with 0.2 to 0.4 of a cubic centimeter of material representing three or more pooled cultures grown for twelve days in the incubator. Another series of rabbits was injected intravenously with from 3 to 5 c.c. of similar materials. Guinea pigs received intraperitoneal injections of from 3 to 5 c.c. of identical cultures, and finally a group of mice was inoculated into the peritoneal cavity with from 1 to 2 c.c. of a suspension of culture. The animals were kept under observation for one month and longer.

The results of all these inoculations were entirely negative both as to immediate and subsequent effects. At no time were there any symptoms or signs characteristic of an infection of the nervous system, the blood stream or the peritoneal cavity. The outcome was different when similar inoculations were practiced with cultures of streptococci or staphylococci obtained from various sources. The usual types of infection resulted and were generally fatal. In the rabbit, particularly, within twenty-four to thirty-six hours symptoms and signs of meningitis and abscess formation developed. At autopsy the gross findings were characteristic and the organisms were present in abundance at the site of inoculation and in the blood stream.

From these experiments it may be observed that the organism isolated and cultivated from poliomyelitic tissue, while capable of infecting and inducing experimental poliomyelitis in the monkey, is without effect in rabbits and smaller animals. These results, directly opposed to the findings of Rosenow and others, confirmed the belief that we were not dealing with an ordinary streptococcus or bacterium. In the monkey, moreover, inoculation of this culture was always identified with the typical incubation period of from six to eight days during which symptoms and signs were uniformly absent. Again, this result rules out a streptococcus or other organisms described by Rosenow and subsequent workers.

#### VRUCIDAL POWER OF SERUMS FROM CONVALESCENT MONKEYS INOCULATED WITH CULTURES, AND RESISTANCE TO REINOCULATED POLIOMYELITIC VIRUS

Tests were made to compare the immunity response that is known to occur in typical experimental poliomyelitis with the results observed in monkeys that had been successfully inoculated with the culture. The question was concerned with whether or not an infection induced by the organisms would protect the animal against inoculation with the regular poliomyelitic virus. It has been shown that recovery from poliomyelitis induced by the filtered virus causes a state of resistance in monkeys. This is indicated by the power of the blood serum from convalescent monkeys to neutralize the virus filtrate<sup>10</sup>. Such animals also become insusceptible to reinoculation with virus<sup>46</sup>. Attempts were therefore made to determine whether monkeys in the convalescent stage following injection of cultures exhibited protection by either of these two reactions.

*Neutralization in Vitro*—For this test the serums were taken from four monkeys. These had developed typical symptoms and paralyses following inoculation with cultures. Two monkeys had received cultures in the fourth subplant, and two other monkeys, the eleventh and the thirteenth subplant, respectively. Serum samples were withdrawn six to fourteen and one-half weeks after the initial symptoms and paralysis had appeared. The physical condition of two of these animals was fair, in two others there was advanced tuberculosis and marked cachexia.

*Experiment 13*—The tests for neutralization were made by mixing 0.2 c.c. of a 5 per cent emulsion of poliomyelitis virus, glycerolated five days at 4° C., with 0.2 c.c. of each monkey serum, incubating for one hour at 37.5° C., and keeping for nine hours at 4° C. These mixtures which had been previously made up to a volume of one c.c. with sterile physiologic salt solution, were injected intracerebrally, under full ether anesthesia, into four normal *Macacus rhesus* monkeys. Two normal control animals received the virus suspension alone in the amounts of 0.1 c.c. and 0.05 c.c., respectively. This control material was subjected to the identical preliminary incubation and ice box fixation as were the serum virus mixtures.

*Macacus rhesus 78* February 24, 1932, intracerebral inoculation of mixture of 0.2 c.c. of 5 per cent virus suspension and 0.2 c.c. serum taken from Monkey 43, fourteen and one half weeks after initial symptoms of poliomyelitis induced by a culture in the fourth subplant that had been passed through a second monkey. No symptoms, remained well.

*Macacus rhesus 79* February 24, 1932, received mixture of virus suspension and serum taken from Monkey 58, on the forty eighth day following first symptoms of poliomyelitis induced by inoculation of a culture in the eleventh subplant. No symptoms, remained well.

*Macacus rhesus 80*—February 24, 1932, was injected into the cerebral hemisphere with a mixture of 0.2 c.c. of virus suspension and serum taken from Monkey 59, eight weeks after first symptoms of poliomyelitis following inoculation of a culture recovered from a monkey that had been injected with the original organisms in the fourth subplant. The animal remained free from symptoms until March 12, 17 days after the inoculation, when weakness of the right arm and both

legs were noted. The neck and back muscles were only slightly involved. Recovery of function progressed rapidly. Monkey 59 from which the serum was taken developed weakness of the left deltoid on the twelfth day following inoculation of the culture, and definite paralysis of the member four days later. There were no other effects and the recovery was rapid with complete restoration of function in the arm. The infection was very mild.

*Macacus rhesus 81* February 24, 1932, intracerebral injection of 0.2 cc serum and 0.2 cc virus suspension. Serum was taken from Monkey 69 on the forty first day following initial symptoms and paralysis induced by inoculation with a culture in the thirteenth subplant. This monkey was in very poor condition on this date and died on the following day. No symptoms, remained well.

*Macacus rhesus 82* (Control) February 24, 1932, received 0.1 cc of a 5 per cent virus suspension into the cerebral hemisphere. March 1, bilateral ptosis, excitability, head tremor, and right facial paralysis were noted. March 2, the animal was prostrate and unable to move arms and legs.

*Macacus rhesus 83* (Control) February 24, 1932, intracerebral inoculation of 0.05 cc of 5 per cent virus suspension. First symptoms were noted March 1, excitement, ruffled fur, and started cry. March 4, paralysis of arms and legs, ataxia and paralysis of muscles of trunk were observed. March 5, prostrate, and unable to move extremities.

**Protection Tests**—Three monkeys were available of the four which had supplied the serums used in the neutralization experiment. These animals were now tested for protection against poliomyelitic virus introduced directly into the brain. A 5 per cent emulsion was prepared from the cord and brain of the same active virus that was used in the preceding tests. The material differed only with respect to an additional storage of three days in glycerol at 4° C. The dose of virus used was considerably larger, namely 0.3 cc to 0.4 cc.

*Monkey 43* Convalescent fourteen and one half weeks. February 27, 1932, intracerebral inoculation of 0.3 cc of a 5 per cent virus suspension, glycerolized eight days at 4° C. No symptoms, remained unchanged.

*Monkey 58* Convalescent forty eight days. February 27, 1932, intracerebral injection of 0.4 cc virus suspension. No symptoms, remained unchanged.

*Monkey 59* Convalescent eight weeks. February 27, 1932, intracerebral inoculation of 0.3 cc suspension of virus. No symptoms, no change.

*Monkey 75* (Control) Normal animal. February 27, 1932, inoculated 0.3 cc of the same virus suspension into the left cerebral hemisphere. No symptoms developed until March 5, when the animal showed lessened activity and sat quietly in cage. March 7, weakness of arms, deltoid muscles and back were noted, inability to climb and fatigability were observed. There was a suggestive right facial paralysis and ptosis of right eyelid.

The results are summarized in Table III.

**Comment**—These tests are significant in having demonstrated the neutralizing power for virus suspensions of serum taken from monkeys that have been inoculated with a culture of organisms capable of inducing the picture of typical poliomyelitic infection. These convalescent monkeys have likewise resisted an inoculation of poliomyelitic virus directly into the brain.

It may be deduced from this experiment that inoculation of the culture has caused in monkeys a resistance to poliomyelitic infection of the same nature and to a similar degree as that which is known to follow an infection with the ordinary virus.

Resistance to poliomyelitic infection has been demonstrated by means of this recognized test for serum neutralizing power and for protection against the effects of virus. It should be noted that the conditions imposed by this experiment have

TABLE III

NEUTRALIZATION TESTS WITH SERUM AND PROTECTION TESTS IN CULTURE INFECTED MONKEYS

NO	DATE	MATERIAL USED	AMOUNT CC	VIRUS AMOUNT SUSP CC	RESULT
78	2/24/32	Serum, culture monkey, 14½ weeks convalescence	0.1	0.1	No symptoms
79	2/24/32	Serum, culture monkey, forty eighth day convalescence	0.1	0.1	No symptoms
80	2/24/32	Serum, culture monkey, 8 weeks convalescence	0.1	0.1	Mild symptoms, seventeenth day
81	2/24/32	Serum, culture monkey, forty first day convalescence	0.1	0.1	No symptoms
82	2/24/32	Control	-	0.1	Typical poliomyelitis, sixth day
83	2/24/32	Control	-	0.05	Typical poliomyelitis, seventh day
43	2/27/32	Culture monkey, 14½ weeks convalescence		0.3	No change
58	2/27/32	Culture monkey, forty eighth day convalescence		0.4	No change
59	2/27/32	Culture monkey, 8 weeks convalescence		0.3	No change
75	2/27/32	Control		0.3	Poliomyelitis, seventh day

been purposefully made more stringent than are demanded by the established technic. A suspension of virus instead of the Berkefeld N filtrate has been used, the larger control monkeys have received half or less than half of the amount of virus inoculated into the test animals, the proportion of serum to the virus was 1:1 instead of the usual 10 to 100:1, finally the period of incubation and ice box fixation of the serum-virus mixtures was one hour and nine hours as against the usual periods of two hours and sixteen hours, respectively. Notwithstanding these rigorous requirements, the serum from three monkeys completely neutralized the larger doses of virus and that of the fourth animal delayed the appearance of a mild infection 12 days beyond the period in the controls. These were already paralyzed and prostrate on the sixth day after inoculation.

## DISCUSSION

The results of the experiments described in the foregoing protocols indicate that poliomyelitis can be induced in monkeys by inoculation with certain organisms that have been cultivated from poliomyelitic nervous tissues. Successful inoculations in monkeys have resulted with visible cultures in the fourth to the eleventh, and also in the thirteenth subplants representing an extraordinary dilution of the original material cultivated. Koch's postulates have been fulfilled in all details including the recultivation of the identical organism with which poliomyelitis could again be reproduced in the experimental animal. Such recaptured "virus" possessed the original characteristics of filterability and pathogenicity.

This was shown by successful inoculations with Berkefeld filtrates prepared from the nervous tissues of monkeys that had been infected with a culture

The theoretical possibility of original active virus being carried along with transfers of the culture material appears to be remote. Dilutions of the material as high as  $2 \times 10^{-27}$  would tend to exclude this and to confirm the belief that actual independent multiplication of an organism has occurred. The virulence of the culture could not be ascribed to the influence of virus as such upon remote subplants

There remain for consideration also the theoretical possibility of adsorption of virus to the bodies of these organisms, and further, the multiplication of invisible virus in the tissue constituting the culture medium

With regard to the first point, there is no explanation to account for the infectivity of the cultures except on the basis of their activity. If it were true that an invisible virus might be a constant companion of bacteria through an indefinite number of transfers, then there is no justification for the existence of bacterial etiology of disease. In yellow fever studies it has indeed been proved that a commonly associated organism cannot act as a vector for the virus.<sup>47</sup> As to the second point under consideration, the culture medium does not contain living tissue, and hence cannot be regarded as a possible nidus for the multiplication of a virus in its invisible state apart from the visible organisms that thrive in the sterilized tissue

Of interest also in connection with the hypothetical survival of original virus in the cultures is the observed viability of the organisms for long periods of time at incubator temperature. From such cultures recent subplants retain infective power for monkeys

The relation of streptococcal organisms to poliomyelitis has again been shown to be nonexistent. In the experiments reported here, only the disease recognized as experimental poliomyelitis in the monkey has been considered. Rabbits and smaller animals in contradistinction to the monkey, failed to develop any symptoms or signs of this disease when they were inoculated with the culture described in the foregoing protocols

Although successful cultivation of an anaerobic organism from poliomyelitis virus was accomplished by means of the EB medium in conjunction with the VB medium for subplants, further study will be necessary to establish the possible dependence of either one upon the other. For the recultivation of organisms from monkeys that had been successfully inoculated with the culture, the VB medium by itself generally was found satisfactory. However, the questions of adaptation of the strain, its multiplication, and maintaining of its virulence and infective power may be modified profoundly by the sequence of events taking place in the different culture mediums over varying periods of time. These matters must be left for future investigation as well as those recognized variable factors inherent in the culture mediums employed, undoubtedly a set of circumstances which affect the outcome of attempts to cultivate the virus of poliomyelitis

#### SUMMARY AND CONCLUSIONS

Bacteriologic cultivation experiments were attempted with Berkefeld N filtrates prepared from the nervous tissues of monkeys inoculated with poliomyelitic virus and developing typical experimental poliomyelitis. Seven strains of virus



were studied in special culture mediums to be designated as EB and VB medium free from living tissue and containing essentially minced sheep brain in a veal infusion free from peptone

Positive cultures were obtained from virus filtrates and the transfers from the original cultures have yielded successful growth in series. At the present time these organisms are in the twentieth subplant and the original cultures have been found viable after an incubation period of more than twenty-two weeks at 37.5° to 38° C.

The organism is a strict anaerobe and grows in an atmosphere of reduced oxygen tension developed in the culture medium. Stains best with Wright stain and in later subplants and older cultures it appears to be faintly to moderately gram-positive. Under a magnification of 1500 to 2000 diameters the organisms appear as minute ovoid bacteria ranging in size from the lowest limit of visibility to 0.05-0.1 or 0.2 of a micron, and occur in irregular clusters, singly, in pairs, very rarely in short chains, and in densely packed masses. The bacterial bodies are especially numerous in the films of particulate brain tissue found in the medium, for which they exhibit a marked preference. A sheath-like envelop appears to surround the organism, favoring the formation of irregular masses.

Cultivation of the organism on the surface of a solid medium has also been successfully accomplished from subplants under strict anaerobiosis by means of the Ohtsky-Boez apparatus. The culture medium devised for this purpose contained VB sheep brain in a base of buffered veal infusion agar, free from salt and peptone, and adjusted to pH 7.6. The presence of abundant moisture was found essential for surface growth.

Inoculation in monkeys of organisms obtained from washed and unwashed cultures isolated from poliomyelitic tissues in VB medium caused clinical symptoms and signs and typical pathologic effects characteristic of experimental poliomyelitis. Berkeley N filtrates as well as suspensions of nervous tissue from such infected animals were capable of inducing the identical infection in the normal monkey. The recaptured "virus" possessed the original characteristics of filterability, pathogenicity, and recultivation in an artificial medium.

Successful experiments up to the present time have been accomplished with these cultures in the third, fourth, eighth, ninth, eleventh, and thirteenth subplants, representing a dilution of approximately  $2 \times 10^{-7}$  to  $2 \times 10^{-27}$  of the original material cultivated. This, it will be noted, was 0.1 cc. of a 5 per cent saline Berkeley N filtrate prepared from poliomyelitic nervous tissue. Parallel experiments in which heated cultures were used for inoculation into monkeys resulted negatively. Materials derived from culture tubes that contained no demonstrable organisms were also without effect. Additional control experiments included material from normal monkeys, and from the tissues of herpes, encephalitis and neurovaccinia. All these, as well as the uninoculated culture medium by itself gave negative results.

Attempts to recultivate the typical organism from monkeys experimentally infected with the culture and developing poliomyelitis, were successful with Berkeley N filtrates and suspensions prepared from the nervous tissues of such animals. With such recaptured virus cultures the typical picture of experimental poliomyelitis was again induced in normal monkeys. Successful transmission of the

disease from these animals to normal ones was again accomplished by means of Berkefeld N filtrates and suspensions of nervous tissues

The inoculation of rabbits, guinea pigs, and mice with the culture has resulted negatively. Direct injection of massive doses into the brain, or into the blood stream, or peritoneal cavity was uniformly without effect.

Film preparations with material taken at autopsy from the site of inoculation and from the surface and deeper layers of the cord and brain of monkeys were uniformly negative for organisms. Inoculation of such materials into ordinary culture medium gave negative results. The same observations were made in rabbits, guinea pigs and mice.

Serums taken from monkeys that have been inoculated with the culture of organisms capable of inducing the picture of typical poliomyelitic infection were found to possess neutralizing power for active virus suspensions. Such convalescent monkeys likewise resisted an inoculation of poliomyelitic virus into the brain. The resistance to poliomyelitic infection induced by the inoculation of the culture was the same as that which is known to follow an infection with the filtered virus or its suspension.

Filtration experiments with the cultures during certain stages of growth suggest that ultramicroscopic forms may be capable of developing into visible bodies in the culture medium.

The cycle of poliomyelitis has been demonstrated in monkeys inoculated with cultures. The organism has been developed from an invisible to a visible state and the typical disease induced in the experimental animal, in the brain and spinal cord of which the organism again returns to the invisible filterable stage, but retains its power to infect normal monkeys.

It appears from these studies that the poliomyelitic virus has multiplied *in vitro* in a culture medium containing nonliving brain tissue. The organism causes pathologic effects that can be augmented by successive passage of the culture material through a series of monkeys. Notable in this respect is the shortening of the incubation period.

The organism exhibits a marked resistance to the action of 50 per cent glycerol over long periods of time.

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# RESPIRATORY METABOLISM AND PULMONARY VENTILATION IN PULMONARY TUBERCULOSIS\*†

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THERE appear in the literature from time to time reports of increased basal metabolism in tuberculosis although the majority of workers have found the metabolism to be approximately normal except in the presence of fever. Can these conflicting reports be due to differences in the amount or character of the disease other than fever, or are the high values only reports of basal metabolism which was not really basal?

It is very easy to obtain high rates and to be misled into believing the results to be basal because the patient was quiet and the pulse and respiration stationary. Only repeated tests will prove that the first ones were too high and that the patients were not sufficiently relaxed to give the minimal rate.

Pulmonary ventilation has been observed to be higher in tuberculous subjects than in normal subjects, and one cause to which it has been attributed is lowered vital capacity. This work was undertaken in an attempt to determine whether the basal metabolism in a series of tuberculous patients could be related either to the extent or to the intensity of the disease and to determine whether this observed increase in pulmonary ventilation is the result simply of the amount of the destruction of lung tissue, or whether it is in some way more closely related to the toxic condition of the subject. In other words, is it related to the extent or to the progression of the disease?

The study also included observations on the relative increase in the ventilation as the oxygen consumption was raised. This increase could not be too large due to the limited size of the spirometer available, hence the respiratory exchange after food was chosen for study.

It was the plan when this problem was begun to study the pulmonary ventilation and oxygen consumption (from which the basal metabolism could be calculated) in both toxic and nontoxic cases, and in the latter to relate each to the extent of involvement. Unfortunately, due to lack of time, only studies on nontoxic cases were carried out. Consequently, this is a report of studies made of the pulmonary ventilation and oxygen absorption in the basal condition and again after breakfast, of nontoxic cases of pulmonary tuberculosis and similar studies upon a series of normal subjects.

## TECHNIC

A Benedict-Roth metabolism machine and a Haldane gas analyzer were used for the determinations. The Roth was used both as a metabolism machine and also, by making a few simple adjustments, as a simple spirometer for the collection of expired air.

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The usual procedure for basal metabolism was observed. The ambulatory patients, without food since the previous evening, came to the metabolism room and rested on a cot for three-quarters of an hour, and the infirmity patients, also without food for twelve to fourteen hours, rested quietly in their rooms for half an hour. The test was not begun until pulse and respiration had reached a constant level, and during the test the rate and character of the respiration were noted, as it is essential for accurate observations that they do not change when expiration into the spirometer is taking place.

During the first part of the test, a determination was made of the oxygen consumption, as in a regular basal metabolism test, for a period of seven to eight minutes. The second part consisted of a collection of expired air. As the spirometer was too small for a continuous collection of several minutes, after approximately one minute (time noted exactly in seconds in each instance) a three-way valve was turned, at the end of an expiration, so that the subject was breathing out into the room. Readings of the volume of expired air in the spirometer and the time of collection were recorded, a sample of the expired air was collected in a sampling tube as part of the composite sample for later analysis, and the spirometer was emptied. This procedure required about thirty seconds. Then the valve was turned again at the end of an expiration allowing the subject to expire into the spirometer and a second collection was made. This procedure was repeated eight or ten times. The patient then had breakfast, returned for a rest of three quarters of an hour and repeated the experiment which began usually one hour after the end of the meal.

To guard against experimental error, tests were repeated until the subject gave consistent results. Frequently, as is the case in all basal metabolism and respiration work, the first test or two had to be discarded because the results were obviously not correct. Sometimes in the first test the subjects were not completely relaxed, due possibly to slight apprehension about the whole procedure, and their metabolism as a result was too high. Overventilation also, was very likely to occur the first time a subject breathed into the spirometer. Work on two subjects had to be discontinued entirely because they never became sufficiently accustomed to the machine to breathe normally when connected with it.

The composite sample of expired air, which contained equal parts of each spirometer of expired air, was analyzed for both  $\text{CO}_2$  and  $\text{O}_2$ . Duplicate analyses were always made and the  $\text{CO}_2$  required to check within 0.04 per cent (and usually did within 0.02) and the  $\text{O}_2$  to check within 0.05 per cent. Analyses of outside air were made regularly, almost daily, to check the machine.

If there was a difference of more than 5 c.c. per minute between the oxygen as determined in the first part of the test and as determined by the analysis of expired air, the volume of the latter was corrected to make the two agree since the first was considered the more reliable test, because of the intermittent collection of expired air. When occasionally such a correction was found necessary, it was small, since special care had been taken to see that the nature of the respiration during the observation was normal.

The basal metabolism was calculated on the basis of calories per square meter of surface area and compared with the Dub-DuBois (1917) standards. The calculation of the oxygen absorption and carbon dioxide elimination from the analysis

of expired air was made as described by Bailey (1921) Correction factors were taken from the tables compiled by Carpenter (1924)

### SUBJECTS

The group of subjects studied consisted of eight normals and nineteen tuberculous patients in the sanatorium, eleven of the latter with moderately advanced and eight with far advanced lesions Of the normals, three were medical students, two secretaries, one a laboratory worker, one a teacher, and one a nontuberculous pleurisy patient

The moderately advanced patients (with one exception, Case 19) were ambulant at the time they were studied although subsequently four of them, Cases 9, 11, 13, and 17, had increase in infiltration or reactivation of the disease and were put to bed The duration of the disease had been less than a year in all except Case 14 who had had a recent fresh exacerbation of an old lesion of several years' standing

Of the eight far advanced cases three were bed patients and their disease was of recent origin Of the five ambulatory cases, Cases 20 and 21 had each had tuberculosis for about a year and a half and Case 27 had "cured" several years previously

All the subjects in both groups were afebrile and generally nontoxic at the time they were studied

The average age was only slightly different for the three groups In the normals all subjects were between twenty and thirty-five years of age with an average age of twenty-six and a half The moderately advanced cases were between twenty-one and forty-six years of age with an average of twenty-nine and the far advanced cases between eighteen and thirty-six with an average of twenty-nine years

The tuberculous subjects were classified according to the National Tuberculosis Association classification and in addition on the x-ray findings alone According to the extent of the parenchymatous (Pe) lesion they were divided into stages

MODERATELY ADVANCED				
Case 9	Pe2	L	4r and 6 vs c <sub>2</sub>	
Case 10	Pe2	R	3r and 5 vs, 5r and 6 vs, 7r and 8 vs b <sub>1</sub>	
		L	3r and 5 vs c <sub>1</sub>	
Case 11	Pe2	R	3r and 5 vs b <sub>1</sub>	L 6r and 9 vs c <sub>2</sub>
Case 12	Pe2	R	4r and 7 vs c <sub>2</sub>	
Case 13	Pe2	R	3r and 5 vs a <sub>1</sub>	L 2r and 4 vs, 4 and 6r, 7 and 9 vs c <sub>1</sub>
Case 14	Pe2	R	2r and 4 vs b <sub>1</sub>	L 3r and 6 vs c <sub>1</sub>
Case 15	Pe2	R	2r and 4 vs b <sub>1</sub>	L 3r and 5 vs b <sub>1</sub>
Case 16	Pe2	R	1r and 3 vs and lower ½ b <sub>1</sub>	
Case 17	Pe2	R	4r and 5 vs b <sub>1</sub>	L 4r and 5 vs c <sub>1</sub>
Case 18	Pe2	R	4r and 6 vs c <sub>2</sub>	
Case 19	Pe2	R	3r and 5 vs c <sub>2</sub>	L clav and 3 vs a <sub>1</sub>
FAR ADVANCED				
Case 20	Pe3	R	5r and 7 vs c	L 5r and 7 vs c <sub>2</sub>
Case 21	Pe3	R	6r and 9 vs c <sub>1</sub>	L 3r and 5 vs b <sub>1</sub>
Case 22	Pe3	R	6r and 9 vs b <sub>1</sub>	L 4r and 6 vs b <sub>2</sub>
Case 23	Pe3	R	4r and 6 vs c <sub>1</sub>	L 4r and 9 vs b <sub>1</sub>
Case 24	Pe3	R	5r and 9 vs c <sub>1</sub>	L 5r and 9 vs b <sub>1</sub>
Case 25	Pe3	R	6r and 9 vs c <sub>2</sub>	L 5r and 7 vs b
Case 26	Pe3	R	5r and 9 vs c <sub>2</sub>	L 6r and 9 vs c <sub>1</sub>
Case 27	Pe3	R	4r and 7 vs b <sub>1</sub>	L 4r and 7 vs b <sub>1</sub>

2 and 3, which correspond to the National Tuberculosis Association classification of "moderately advanced" and "far advanced." The side and extent on each side, measuring from the apex down to the specified rib or vertebral spine, are recorded with the intensity of the lesion, a, b, or c. The probability of focal change or activity is expressed in suffixes 0, 1, or 2 for each side.

BASAL METABOLISM

The results of the experiments are grouped in two sets of tables, the first dealing with the respiratory metabolism and the second with the pulmonary ventilation. Tables I, II, and III list the metabolism in calories per square meter of body surface per hour together with the percentage deviation from the normal value according to the Aub-DuBois standards. The values after breakfast, with the percentage increase and also the respiratory quotients are given.

In order to shorten the tables, individual experiments have not been listed. As has been stated previously, tests were made until consistent results were obtained and the first experiments were discarded if the results were higher than that found in subsequent tests. Any other experiments were discarded also if for any reason they were not basal. The results given in the tables are the average result of the consistent tests and the column entitled number of tests, represents the number averaged and not necessarily the number actually made upon the subject.

A survey of the tables reveals the fact that of the 27 cases, 25 had a basal metabolism below the expected value. In the normal group there were no values which were higher than the standard and two subjects had values of -16.5 per cent and -19 per cent. The entire group had an average of -9 per cent which, however, falls within the limits of normal variation even if we retain the narrower limits of  $\pm 10$  per cent.

To make certain that the machine was not giving too low oxygen values, a sec-

TABLE I  
RESPIRATORY METABOLISM OF NORMAL SUBJECTS

SUBJ SEX	NO TESTS	NUTRITIVE CONDITION	EXPECTED CAL /SQ M /HR AUB DUBOIS	FOUND CAL /SQ M PFR HR	B M R	INCR. AFT BREAKFAST PER CENT	R Q
1 F	2	Basal	36.5	33.6	- 7.9		0.89
	1	1 hr aft break		37.5		+11.6	0.91
2 F	2	Basal	37.0	30.9	-16.5		0.73
	2	1 hr aft break		33.1		+ 7.1	0.84
3 F	2	Basal	37.0	34.4	- 7.0		0.71
	2	1 hr aft break		39.3		+14.2	0.87
4 M	2	Basal	39.5	39.1	- 1.0		0.80
	2	1 1/4 hr aft break		43.1		-10.2	0.82
5 M	2	Basal	39.5	35.7	- 9.4		0.86
	2	1 hr aft break		41.6		+16.5	0.85
6 M	2	Basal	39.5	32.0	-19.0		0.90
	2	1 hr aft break		37.3		+16.5	0.83
7 F	2	Basal	37.0	33.9	- 8.4		0.79
	2	1 hr aft break		33.1		+12.4	0.80
8 F	2	Basal	37.0	35.9	- 2.8		0.76
	2	1 hr aft break		39.8		+10.8	0.88
Average		Basal	37.6	34.4	- 9.0		0.81
		1 hr aft break		38.7		+12.4	0.86



and portable apparatus of a different make was procured and with a subject thoroughly accustomed to the metabolism tests, alternate determinations were made, first on one machine and then on the other. Four tests, two on each machine, made within the course of an hour, checked within 1.5 absolute per cent. Hence, there is every reason to believe that the low metabolism values are absolutely correct.

TABLE II  
RESPIRATORY METABOLISM OF MODERATELY ADVANCED TUBERCULOUS SUBJECTS

SUBJ SEX	NO TESTS	NUTRITIVE CONDITION	EXPECTED CAL /SQ M /HR AUB DUBOIS	FOUND CAL /SQ M PER HR	B M L	INCR AFT BREAKFAST PER CENT	P Q
9 F	2	Basal	37.0	30.0	-18.9		0.84
	2	1¾ hr aft break		35.1		+17.0	0.86
10 M	2	Basal	39.5	36.0	-8.8		0.78
	2	1 hr aft break		39.2		+8.9	0.89
	1	1½ hr aft break		38.9		-8.0	0.90
11 F	1	Basal	37.0	36.1	-2.4		0.83
	1	1 hr aft break		38.1		+5.8	0.92
12 M	1	Basal	39.5	40.7	+3.0		0.83
	3	1 hr aft break		41.7		+9.8	0.87
	1	1½ hr aft break		42.2		+3.6	0.85
13 F	2	Basal	37.0	34.0	-8.2		0.82
	2	1 hr aft break		45.2		+32.9	0.86
	2	2 hr aft break		40.4		+18.8	0.93
14 M	1	Basal	38.5	37.0	-3.9		0.87
	2	1 hr aft break		43.9		+18.6	0.86
15 M	2	Basal	39.5	34.1	-13.9		0.83
	2	1 hr aft break		37.9		+11.1	0.92
	1	2½ hr aft break		36.1		+5.8	0.88
16 F	2	Basal	36.0	36.5	+1.4		0.82
	2	1 hr aft break		40.7		+11.5	0.94
	1	1½ hr aft break		38.0		+4.1	0.81
17 M	2	Basal	39.5	33.5	-15.3		0.82
	3	1 hr aft break		39.0		+16.4	0.85
18 M	2	Basal	39.5	34.7	-12.1		0.83
	2	1 hr aft break		42.2		+22.8	0.82
19 M	2	Basal	39.5	31.9	-19.2		0.83
	2	1 hr aft break		39.2		+22.9	0.88
Average		Basal	38.4	34.9	-8.9		0.83
		Basal minus No. 9	38.6	35.4	-7.9		0.83
		1 hr aft break		41.0		+16.2	0.88

In the group of moderately advanced cases there were only two positive values, one of +3 per cent and one of -1.4 per cent while there were five with values below -10 per cent. The average for the group was -7.9 per cent.

Even in the far advanced group there were no high rates. Three of the eight cases had values below -10 per cent and the average was -8.0 per cent.

Of the nineteen tuberculous patients there were three whose basal metabolism was between -15 per cent and -20 per cent, five between -10 per cent and -15 per cent and nine between 0 per cent and -10 per cent making seventeen in all that were below the expected value.

Lack of normal muscular activity over a considerable time fails to explain the low values because several cases with low metabolism had been as active as usual only a few weeks prior to the tests.

TABLE III  
RESPIRATORY METABOLISM OF FAR ADVANCED TUBERCULOUS SUBJECTS

SUBJ SEX	NO TESTS	NUTRITIVE CONDITION	EXPECTED CAL/SQ M /HR. AUB DUBOIS	FOUND CAL/SQ M PFR HR.	B V R	INCR AFT BREAKFAST PER CENT	R Q
20 M	2	Basal	39.5	34.3	-13.1		0.78
	2	1 hr aft breakfast		37.1		+8.2	0.86
21 M	3	Basal	39.5	37.2	-5.8		0.84
	4	1 hr aft breakfast		47.7		+28.2	0.89
	1	1½ hr aft breakfast		42.4		+13.9	0.87
22 F	2	Basal	36.5	35.5	-2.7		0.82
	2	1 hr aft breakfast		45.3		+27.6	0.95
	1	1½ hr aft breakfast		43.8		+23.1	0.88
23 F	2	Basal	36.5	31.8	-12.9		0.83
	2	1 hr aft breakfast		35.9		+12.9	0.88
24 M	2	Basal	39.5	36.6	-7.3		0.87
	2	1 hr aft breakfast		42.5		+16.1	0.94
25 M	2	Basal	39.5	38.5	-2.5		0.78
	2	1 hr aft breakfast		45.8		+19.0	0.85
26 F	3	Basal	38.0	36.1	-5.0		0.83
	3	1 hr aft breakfast		39.2		+8.6	0.87
27 M	2	Basal	39.5	33.7	-14.7		0.86
	2	1 hr aft breakfast		38.6		+14.5	0.88
Average		Basal	38.6	35.5	-8.0		0.83
		1 hr aft breakfast		41.5		+16.9	0.89

Thus in these studies of afebrile nontoxic cases there was not the slightest evidence of an increased basal metabolism in either group. The rate in the series of far advanced cases was equally as low as for the moderately advanced series and as low as in a similar group of normal subjects which makes it appear that basal metabolism in the tuberculous person bears no relationship to the *amount* of the involvement, i. e., to extent of lesion.

Broek and Haskins (1927) also obtained low basal metabolism values for several cases which correspond with the values reported for this work, a -14 per cent for the average of three patients studied over several months during the summer when our cases were studied.

The majority of workers find the basal metabolism to be approximately normal for minimal tuberculous subjects. Among the more recent workers holding that view are Lanz (1925), Giegler (1927), Frank and Safarik (1928), Salus and Adler (1928), and McMahon and Klein (1929). McBayer (1921), on the other hand, finds in 61 per cent of his "incipient" tuberculous subjects (22 cases) a basal metabolic rate greater than normal, a percentage which is even slightly higher than for a group of 21 advanced cases in which 57 per cent were above normal.

Concerning the metabolism in advanced cases there is more disagreement. For example, Gekler and Weigel (1926) from their study of eighty cases conclude that the toxemia of tuberculosis does not increase the basal metabolic rate and that increased rates are to be associated with hyperthyroidism, while Giegler (1927) with one hundred thirty cases reports normal values only for minimal tuberculosis. With higher values for advanced cases, the rate corresponding to the grade of activity.

Cordier (1923) reports normal basal metabolism in afebrile cases, but Hyge (1928) finds increased rates in the third stage even when afebrile, although he

states that when the patient is in good condition clinically the metabolism is usually normal

McCann and Barr (1920) in their careful study of tuberculous patients in the calorimeter of the Russell Sage Institute found basal values for ten cases to be between -3 per cent and +15 per cent with an average value of +8 per cent. With fever, however, the metabolism was increased, and, with a rectal temperature of  $40^{\circ}\text{C}$ , the authors find it may go as high as 30 per cent above normal.

Williamson (1929) reports average values of +23 per cent and +18 per cent for two groups with pyrexia or other toxic symptoms and -0.5 per cent and -1.0 per cent for apyretic groups. McMahon and Klein (1929) with a group of forty active cases who had fever during the day but were afebrile in the morning when the tests were made give the following average figures for basal metabolism, incipient cases, +3.9 per cent, moderately advanced cases, +13 per cent, and far advanced cases, +21 per cent.

The greatest increases are reported by Grafe (1920), who in seven of ten afebrile acute tuberculous subjects found increases of from 20 to 36 per cent, and in three febrile (over  $39^{\circ}\text{C}$ ) cases, increases from 50 to 75 per cent. His values undoubtedly are not basal. The determinations were made in a Jacquet chamber over periods several hours in length and such results cannot be compared with short periods during which the subject can remain absolutely quiet and relaxed.

#### INFLUENCE OF FOOD

The chief interest in the study of the metabolism after breakfast was not in the specific dynamic action of the food, but rather in the relationship between the increase in the oxygen consumption and the increase in the pulmonary ventilation. This relationship will be discussed later in the section on pulmonary ventilation.

In these experiments, since it was not feasible to have each subject given the same breakfast and in equal amounts, the results upon each individual cannot be compared one with the other. The average values for the groups are, however, not without interest.

In the normal group the metabolism, one hour after the meal, showed increases varying from 7.1 per cent to 16.5 per cent with an average of 12.4 per cent. The average respiratory quotient rose from 0.81 to only 0.86 due, undoubtedly, to the relatively large amounts of protein and fat in the meal.

The moderately advanced group showed wide variations from 5.8 per cent in one case to 32.9 per cent in another. The average increase was 16.2 per cent and was practically the same for the men and the women. The respiratory quotient rose from 0.83 to 0.88.

The individual variation within the group of far advanced cases was nearly as great, 8.2 per cent to 28.2 per cent, and the average increase is the same as for the moderately advanced group, i. e., 16.9 per cent. The respiratory quotient also shows the same rise, 0.83 to 0.89.

Since the amount of food eaten in each case was not controlled, it is impossible to tell whether or not this slightly greater increase which was observed in the tuberculous groups is a real difference due to the disease. It is not great enough to be very significant at any rate, and too, both McCann (1921) and Salus and Adler

(1928) report a normal response to food in the tuberculous. The latter authors found no relationship between specific dynamic action and activity in the lungs and the writer's results would tend to bear out this conclusion.

#### PULMONARY VENTILATION

In comparing the pulmonary ventilation, oxygen consumption and carbon dioxide production of one group of subjects with another, there arises the difficulty of individual variations due to size, sex, and age. It is definitely known that the oxygen and carbon dioxide are affected by all three factors and it is most probable that the ventilation is likewise influenced. This is borne out by the findings of several workers (Krogh and Landhaid, 1913, Boothby, 1915, Pearce, 1921) that pulmonary ventilation varies directly with the oxygen consumption.

In computing basal metabolism, this difficulty is eliminated by expressing the calories on the basis of surface area of the body and comparing them with standards which take account of differences due to sex and age. But for pulmonary ventilation we have no such standards. In this work the age factor may be eliminated since the average age of each group was nearly the same. The results may then be expressed on the basis of surface area or they may be arranged according to sex, with a weighted average depending upon the number of males and females in the group.

The ventilation values in this work have been calculated by both these methods and, curiously enough, the variations for the three groups of subjects were of the same order of magnitude whichever way they were expressed. This is probably

TABLE IV  
PULMONARY VENTILATION OF NORMAL SUBJECTS

SUBJ SEX	NO TESTS	NUTRITIVE CONDITION	EXPIRED AIR LITERS/MIN	INCR. %	CO <sub>2</sub> O CC MIN	INCR IN O <sub>2</sub> %	PULSE	RESP
1 F	2	Basal	5.84		160/179		50	13
	1	1 hr aft break	6.27	7.4	182/199	10.0	56	16
2 F	2	Basal	4.08		120/164		68	16
	2	1 hr aft break	3.97	-2.8	147/177	7.9	70	16
3 F	2	Basal	4.86		125/176		74	12
	2	1 hr aft break	5.57	14.6	175/201	14.2	82	14
7 F	2	Basal	5.88		161/203		75	14
	2	1 hr aft break	6.13	4.3	180/226	11.3	79	16
8 F	2	Basal	5.55		155/203		81	14
	2	1 hr aft break	6.60	18.9	198/227	11.4	88	15
Average for F		Basal	5.24		144/185		70	14
		1 hr aft break	5.70	8.5	176/205	10.9	75	15
4 M	2	Basal	5.61		209/262		52	8
	2	1 1/4 hr aft break	6.02	7.3	238/290	10.7	52	10
5 M	2	Basal	6.81		220/255		52	11
	2	1 hr aft break	7.51	10.3	252/297	16.4	64	13
6 M	2	Basal	5.99		180/199		50	11
	2	1 hr aft break	6.30	5.2	204/230	15.5	58	12
Average for M		Basal	6.14		203/238		52	10
		1 hr aft break	6.61	7.6	231/272	14.2	58	12
Mean		Basal	5.69		173/211		61	12
		1 hr aft break	6.16	8.1	203/238	12.6	66	13

TABLE V  
PULMONARY VENTILATION OF MODERATELY ADVANCED TUBERCULOUS SUBJECTS

SUBJ SEX	NO TESTS	NUTRITIVE CONDITION	EXPIRED AIR LITERS/MIN	INCR. %	CO <sub>2</sub> /O <sub>2</sub> CC MIN	INCR IN O <sub>2</sub> %	PULSE	RESPI
11 F	1	Basal	5.31		168/202		84	11
	1	1 hr aft break	6.28	18.3	197/214	5.9	84	12
13 F	2	Basal	5.24		168/204		76	18
	2	1 hr aft break	6.02	32.1	234/269	31.9	88	20
16 F	2	2 hr aft break	6.70	27.9	222/239	17.1	76	20
	2	Basal	5.18		157/190		72	16
	2	1 hr aft break	6.02	16.2	205/212	11.5	72	16
	1	2 hr aft break	5.16	0.0	160/197	3.7	80	16
Average for F			5.24		164/199		77	15
			6.41	22.2	212/231	16.4	81	16
10 M	2	Basal	5.02		175/217		70	11
	2	1 hr aft break	5.49	9.4	209/236	8.8	80	12
12 M	1	1½ hr aft break	5.38	7.1	211/234	6.0	80	11
	1	Basal	7.46		235/282		68	18
	3	1 hr aft break	8.38	12.3	272/310	9.9	76	19
14 M	1	1½ hr aft break	8.08	8.3	249/293	3.7	72	16
	1	Basal	6.52		211/243		68	9
15 M	2	1 hr aft break	7.00	7.4	247/284	16.9	72	14
	2	Basal	5.60		173/209		64	12
17 M	2	1 hr aft break	6.78	21.1	216/232	11.0	64	12
	1	2½ hr aft break	6.20	10.7	192/220	5.3	64	12
18 M	2	Basal	5.72		176/216		80	15
	3	1 hr aft break	6.61	15.6	215/253	17.1	80	17
19 M	2	Basal	5.56		186/226		78	6
	2	1 hr aft break	6.06	9.0	225/275	21.7	84	4
19 M	2	Basal	5.37		177/215		74	13
	2	1 hr aft break	6.23	16.0	232/264	22.7	86	11
Average for M			5.89		190/230		72	12
			6.65	13.0	231/264	15.4	77	13
Mean			5.57		177/215		75	13
			6.53	17.6	222/249	15.9	79	14

pure coincidence for this series. Since it was simpler to arrange the values according to sex, that method was adopted and Tables IV, V, and VI give separate averages for the men and for the women, of the pulmonary ventilation, of the percentage of CO<sub>2</sub> in the expired air, of the CO<sub>2</sub> and O<sub>2</sub> in c.c. per minute, as well as of the pulse and respiration rates. At the foot of the tables is given the mean of the two averages.

From the tables it will be seen that, in the basal condition, the pulmonary ventilation varies for women between 4.08 and 5.84 liters per minute, and for men between 5.99 and 6.81 liters, the weighted average being 5.69. In the group of moderately advanced cases the values are nearly identical for the three women, i.e., 5.24 liters, and vary for the men between 5.02 and 7.46. The average for the entire group is 5.57 liters which is even slightly less than that of the normals. In the far advanced group the variation among the women is between 5.42 and 6.22 and among the men between 5.11 and 7.23, the group average being 6.05 liters per minute.

Evidently then, the ventilation, in the basal state, as well as the oxygen consumption, can remain practically normal even with considerable involvement of

TABLE VI  
PULMONARY VENTILATION OF FAR ADVANCED TUBERCULOUS SUBJECTS

SUBJ SEX	NO TESTS	NUTRITIVE CONDITION	EXPIRED AIR LITERS/MIN	INCR %	CO O <sub>2</sub> C C MIN	INCR IN O %	PULSE	RESP
22 F	2	Basal	5.42		160/195		84	12
	2	1 hr aft break	7.08	30.6	232/248	27.2	88	13
	1	1½ hr aft break	6.75	24.5	213/242	24.1	92	11
23 F	2	Basal	6.22		167/202		82	13
	2	1 hr aft break	7.34	18.0	201/229	13.4	86	13
26 F	3	Basal	5.69		158/188		84	13
	3	1 hr aft break	5.83	2.5	175/201	6.8	99	12
Average F		Basal	5.78		162/195		83	13
		1 hr aft break	6.75	17.0	203/226	15.8	91	13
20 M	2	Basal	5.11		158/205		78	13
	2	1 hr aft break	6.02	17.8	188/219	6.8	82	13
21 M	3	Basal	7.23		221/264		83	9
	4	1 hr aft break	9.00	24.5	304/338	28.0	86	11
	1	1½ hr aft break	7.63	5.5	262/300	13.6	80	9
24 M	2	Basal	6.90		207/238		70	13
	2	1 hr aft break	8.02	16.2	258/276	16.0	92	13
25 M	2	Basal	5.43		179/231		116	9
	2	1 hr aft break	6.74	24.1	232/275	19.0	120	11
27 M	2	Basal	6.97		187/217		62	13
	2	1 hr aft break	7.90	13.3	221/249	14.8	84	15
Average M		Basal	6.33		190/231		82	11
		1 hr aft break	7.54	19.2	240/271	16.3	93	13
Mean		Basal	6.05		176/213		83	12
		1 hr aft break	7.15	18.1	222/249	17.1	92	13

lung tissue. The value in the far advanced series is only 6 per cent above that of the normals and is, undoubtedly, within the limits of normal variation. Certainly it is very insignificant in view of the increase which McCann (1921) found of nearly 100 per cent for five toxic cases. His subjects were all in an advanced stage of the disease, much emaciated and with considerable fever during the day although not particularly high in the morning when the observations were made, the average rise above normal being 1.3° F. His average value for five normals was 4.255 liters per minute, which is less than was found in this series of normals, but the average for his tuberculous cases was 8.249 liters, which is 94 per cent above his normal value and 45 per cent above the normal value of this series.

There is then this striking difference in the amount of the pulmonary ventilation between toxic and nontoxic cases. The amount of the involvement was undoubtedly greater in the cases which McCann studied but surely not enough greater to account for the large difference found, since the nontoxic cases, even the ones with lesions sufficiently extensive to place them as far advanced cases, had a normal ventilation.

It is impossible to say whether this difference is due to a greater reduction of vital capacity as McCann suggested or not, since, unfortunately, there are no vital capacity determinations in either series of studies. It is quite possible that this is so because there is considerable evidence indicating that vital capacity becomes progressively lower as the disease advances (Myers, 1925). On the other hand, the lowered vital capacity may be only one of several factors present in the toxic state.

which are affecting the pulmonary ventilation and only further work can give us the answer to that

If we turn now to a consideration of the increase in ventilation following the breakfast, we find considerable variation in all three series, as we should expect with the diet uncontrolled. In the normals, however, the increases never reached the heights that they did in some of the tuberculous subjects. With one normal, Case 2, the respiratory exchange after the meal was slightly less than before, although the oxygen consumption was raised 8 per cent.

As was brought out in the section dealing with the metabolism after breakfast, individual figures cannot be compared because of lack of uniformity of the breakfasts, but the average value for the three series is of some interest, since the average amount of food eaten in each group would probably be approximately the same. We find that the normals increased their ventilation only 8 per cent, while the tuberculous groups increased theirs 18 per cent.

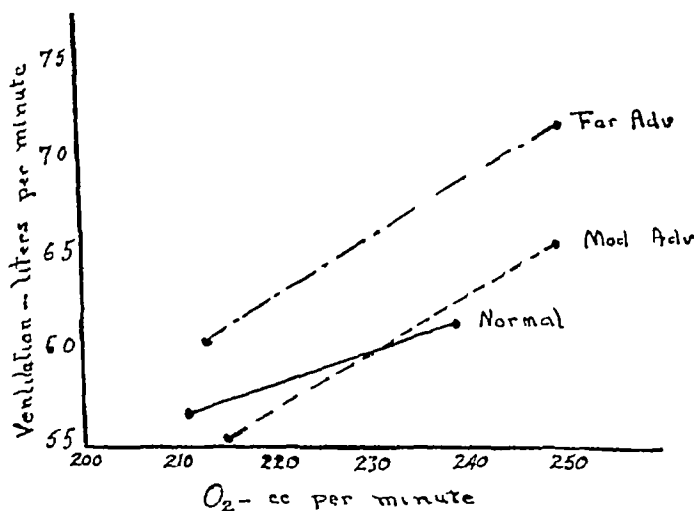


Fig 1

The significant point in this connection is the relative increase in ventilation and in oxygen consumption. The normals, in order to increase their oxygen intake 12.6 per cent, needed to increase their ventilation only 8.1 per cent, whereas the moderately advanced groups in order to increase their oxygen 15.9 per cent needed to increase their ventilation 17.6 per cent, and the far advanced group increased their oxygen 17.1 per cent and their ventilation 18.1 per cent. This is shown graphically in Fig 1, in which the basal and after food values for oxygen are plotted against the corresponding values for ventilation. The slope of the curve for the tuberculous groups is definitely steeper than that for the normal group.

All three groups had a slight rise in pulse rate after breakfast, but the respiratory rate was, on the average, only one point higher than before breakfast.

Thus it appears that a patient may have extensive involvement in both lungs, but, if the disease is of sufficiently low virulence or the resistance of the body is sufficiently high so that no toxic symptoms make their appearance, the lungs are still

able, with their normal amount of ventilation, to supply the body with the necessary amount of oxygen in the basal condition, that is, the minimal requirement of oxygen. This mechanism, however, must be working at nearly its maximum since a small increase in oxygen consumption of only 17 per cent caused in the tuberculous subjects an equal or slightly greater percentage increase in ventilation, whereas the normal subjects increased their ventilation by less than two-thirds the amount of their percentage increase in oxygen.

This corroborates the findings of Williamson (1928) who reported for a series of observations on one normal and on four tuberculous patients the  $O_2$  consumption and pulmonary ventilation values for basal conditions, again at 11 15 A M and at 5 15 P M, after tea. His values for basal  $O_2$  for the eight determinations on the normal varied between 197 and 252 c.c. per minute and the pulmonary ventilation between 4.76 and 6.09 liters per minute. Since we know that the basal metabolism usually varies within rather narrow limits (Lusk 1928), these variations of as much as 28 per cent are rather disturbing, but if we calculate the percentage increases of  $O_2$  and ventilation which he found at 11 15 A M and again after tea at 5 15 P M we find exactly the same relation that the writer finds, namely that, in the normal the percentage increase in pulmonary ventilation is only about two-thirds of the percentage increase in  $O_2$  consumption. His increases were 21 and 35 per cent for the  $O_2$  and 15 and 22 per cent respectively for the ventilation. The tuberculous patients, on the other hand, needed to increase their ventilation slightly in excess of the increase in  $O_2$ . Increases of 15 and 22 per cent in the  $O_2$  called for increases in the ventilation of 17 and 27 per cent respectively.

This is only a small difference, but if we realize that this difference is the result of a rise in  $O_2$  consumption of approximately 20 per cent and that a man walking down the street at a moderate pace can double or more than double his  $O_2$  absorption (Benedict and Muchhauser, 1915), this difference becomes a significant factor in the dyspnea of many tuberculous subjects following the slightest exertion.

#### SUMMARY

- 1 With a Benedict-Roth metabolism machine and a Haldane gas analyzer the respiratory metabolism and pulmonary ventilation were determined before breakfast (basal) and again one hour after breakfast on eight normal and nineteen tuberculous subjects. The latter were composed of eleven with moderately advanced pulmonary lesions and of eight with far advanced pulmonary lesions, but all were afebrile, nontoxic patients and fifteen were ambulant.

- 2 The basal metabolism of the normal group averaged -9 per cent when compared with the Aub-DuBois standards, that of each of the moderately advanced group and of the far advanced group averaged -8 per cent.

- 3 After breakfast, which was not controlled as to amount or character, the increase in metabolism amounted to 12.4 per cent, 16.2 per cent, and 16.9 per cent respectively for the normals, moderately advanced cases and far advanced cases.

- 4 The average pulmonary ventilation in the basal condition was 5.69 liters per minute for the normals, 5.57 liters for the moderately advanced cases, and 6.05 liters for the far advanced cases.

- 5 The increase in ventilation after breakfast was 8.1 per cent, 17.6 per cent, and 18.1 per cent for the three groups.



6 When compared with the increase in oxygen consumption after food, the percentage increase in the ventilation was, in the normals, only two thirds the percentage increase in the oxygen absorption, whereas, in both tuberculous groups, the percentage increase in ventilation was equal to or slightly in excess of the percentage increase in the oxygen absorption

#### CONCLUSIONS

1 In nontoxic, afebrile patients with pulmonary tuberculosis, extent of lesion apparently does not affect basal metabolism. Patients with far advanced pulmonary lesions had a rate which was entirely normal.

2 In nontoxic afebrile patients the pulmonary ventilation, in the basal condition, is normal even in far advanced cases, but when the oxygen requirement is increased the increase in the ventilation in the tuberculous patient is definitely greater than the increase in the normal subject.

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# MONOCYTIC LEUCEMIA

## WITH REPORT OF TWO CASES

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UNTIL rather recently leucemias have been considered to fall into two main groups—myelogenous and lymphatic

Since the case reported by Reschad and Schilling<sup>21</sup> in 1913, there have been a number of reports of "monocytic leukemia," and the concept of this third form seems to be gaining acceptance. Most of the reports have been in German, but a recent (1930) review of the literature with report of two additional cases was published by Dameshek<sup>7</sup> in this country. Since this review additional cases have been described by Bock and Wiede<sup>2</sup>, Failey<sup>8</sup>, Weissenback, Martineau et al<sup>23</sup>, Muenzer<sup>17</sup>, and Lawrence Josey and Young<sup>12</sup>. References are also found in the recent literature to leucemias in which the type cell is so primitive that it cannot be readily classified. For these the term "stem cell leukemia" has been proposed by Hoff<sup>14</sup>. Cases which might fall in this group have been described also by Ewald,<sup>3</sup> Pinkerton,<sup>20</sup> Feller and Risak,<sup>9</sup> and Bykowa.<sup>4</sup> A case of "hemohistioblastosis" by Gosio<sup>10</sup> was not available for review. There seems to be no clear-cut distinction between some of the cases of "monocytic leukemia" and some of "stem cell leukemia." In the former, very primitive cells often dominate the blood picture. The present report has made use of the term "monocytic leukemia," as it was considered morphologically descriptive.

The first case was followed at the Infirmary of the University of Wisconsin, and is presented with the permission of Dr. William A. Mowry, chief physician of the Student Health Department. A second case was found in the records of the State of Wisconsin General Hospital, and is included by permission of the attending physician, Dr. R. C. Blankinship.

### CASE REPORT

CASE 1—H. E. G., a white male university student, aged eighteen, who had appeared at the ambulatory clinic twice early in January, 1930, for treatment of a mild upper respiratory infection, was referred to the Infirmary on January 27, his chief complaint being constant pain and aching in the right thigh, extending from hip to knee, felt both anteriorly and posteriorly, and first noticed the preceding day on arising. No history existed of injury or overuse of the limb. The social background showed a heavy schedule of studies, several hours' work daily as an oil station attendant exposed to extremes of temperature, very irregular meals, and a gradually developing fatigue.

Past medical history: Measles, mumps, whooping cough, chicken pox, scarlet fever, tonsillitis, otitis media, influenza, pneumonia, appendicitis (operated). No recent severe illness.

Family history: Irrelevant.

\*From the Department of Clinical Medicine and Student Health, University of Wisconsin.  
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Physical examination showed a subsiding upper respiratory infection, with nonpurulent nasal discharge and injection of the pharyngeal and nasal mucous membranes. The tonsils were hypertrophied and cryptic. The skin was ichthyotic. The affected leg was painful enough to produce a moderate limp, but showed no tenderness, deformity, limitation of test movements, or abnormal reflexes.

Preliminary diagnosis: Rhinopharyngitis, chronic tonsillitis, and myositis of the right thigh, ichthyosis.

The patient remained in hospital nine days, receiving rest, heat, salicylates, and other analgesics. X ray of the pelvis, sacroiliac and hip joints showed nothing suggestive. The temperature ranged from normal to 100° F, averaging 99.4°. The leucocyte counts varied from 9,100 to 7,800, with neutrophilic leucocytes in proportions of from 76 per cent to 91 per cent.

A subsidence of most symptoms and the patient's desire to resume his school work led to discharge on February 5, but he was readmitted four days later. This time his complaint was almost identical with that of the first admission, but the pain was in the left leg instead of in the right. There was now no pain on the right side whatever.

Again a diagnosis of myositis was made, the etiologic factors being regarded as exposure to extremes of temperature in the presence of a low grade focal infection, probably resident in the hypertrophied tonsils. Further examination, with the discovery of tenderness over the sciatic nerve and definite radiation of the pain from the lower left back well down into the leg, led to a diagnosis of a left sciatic neuritis. Treatment included heat in various forms, galvanism, and anodyne drugs. A search for infective foci resulted in negative roentgenologic findings in teeth, lungs, and paranasal sinuses. Prostatic massage produced no abnormal secretion, the urine showed no abnormalities, blood Wassermann proved negative, and the Mantoux intradermal tuberculin test gave a negative result.

Symptoms gradually decreased from February 12 to February 19, so that the patient's back could be comfortably strapped and walking resumed. At this stage abdominal discomfort, due to what appeared to be mild spastic colitis, called for appropriate therapy, with disappearance of symptoms by February 25. On February 23 occurred a moderate epistaxis, patient saying such bleeding had been common in the past few years.

The patient still complained of some pain, and did not feel well. The tonsils were considered to be a focus of infection, possibly responsible for the myositis, neuritis, and general malaise. After having been postponed one day because of a temperature of 100°, tonsillectomy was performed on February 28, pathologic report on tissue, chronic tonsillitis. Following the operation the tonsillar fossae steadily oozed a small amount of blood, the patient became extremely depressed mentally, and complained of somatic weakness out of all proportion to his observable findings. Eating and sleeping were interfered with, but the throat was not unusually painful at any time.

From the time of his admission the patient's temperature had occasionally reached 99.2° F, but it now began to rise to 100° F or 101° F daily. The pulse was dicrotic and often as rapid as 116. The picture was not that of typhoid fever, but a Widal, blood culture, and stool examination were done. All proved negative. Agglutination tests for B abortus and B tularensis were also negative. Preoperative blood studies had shown: Coagulation time four minutes, hemoglobin 79 per cent, erythrocytes 4,830,000 per c mm, leucocytes 4,250 per c mm, polymorphonuclear leucocytes 59 per cent. On March 6, the eighth day after the operation, the figures were: Hemoglobin 57 per cent, erythrocytes 3,280,000 per c mm, leucocytes 3,900 per c mm., polymorphonuclear leucocytes 15 per cent, lymphocytes 81 per cent, large mononuclear leucocytes 1 per cent, myelocytes 1 per cent, eosinophile leucocytes 2 per cent. Two nucleated and an occasional stippled red blood cell were seen. Differential diagnosis lay between an aleucemic type of acute leucemia, an agranulocytic angina, and the leucopenic phase of infectious mononucleosis. The lack of necrosis in the throat, absence of severe pain, and presence of generalized lymphadenopathy (the latter noted first on March 7, 1930) were all against the agranulocytosis, the type of cell against the latter.

Blood smears which had up to this point been examined by a technician were now submitted to Dr. C. H. Bunting, who reported as follows:

"Smear apparently that of an aleucemic phase of leucemia, diagnosis resting upon abnor-

mal character of cells present. Abnormal cells are of extremely primitive character, possibly even primitive mesenchyme cells, and showing marked tendency to formation of pseudoplatelets.

"The striking cell in the smear is a large cell with round or oval nucleus with close packed chromatin masses and definite large nucleolus. The protoplasm of the cell tends to have a clear border with more granular protoplasm about the nucleus. In some cells a few azurophilic granules are present in the protoplasm.

"The cells are round or oval, and in parts of the smear where the neutrophils are so spread as to measure  $12\ \mu$  in diameter, these cells measure up to  $32 \times 27\ \mu$ , with nucleus of  $15\ \mu$  in diameter."

From this point on the course was typically that of an acute leucemia. Transfusion of 500 cc. of whole blood on two occasions produced fleeting relief of symptoms. The daily temperature range was  $99^{\circ}$  to  $101^{\circ}$  F, the pulse rate between 88 and 96. On March 14 a violent epistaxis occurred, and three days later the gums were hugely swollen and showed the presence of large numbers of Vincent's spirillae and fusiform bacilli, as the throat had done one month previously. On March 20 the heart developed "gallop" rhythm and a faint systolic murmur was heard in the third and fourth left interspaces. Next day occurred a severe chill, the temperature rising to  $103^{\circ}$  F, pulse 120, respiration 20, accompanied by a hard, sharp pain in the right chest. Many purpuric spots appeared on the skin of the abdomen and chest, and in the cubital fossae. Chest findings were indicative of congestion generally, and specific enough to lead to a diagnosis of a small infarct of the right upper lobe, with pleurisy and beginning terminal pneumonia. Next morning there was consolidation of the right lower lobe, the heart was tremendously overactive with a loud systolic and diastolic basal murmur transmitted over the whole precordium. Temperature was  $103.4^{\circ}$  F, pulse 120, respiration 28. Systolic blood pressure was 110, the diastolic not ascertainable. Late that day a loud pleural friction rub became audible at the right base posteriorly. A blood culture made at this time returned negative for all growth. By the morning of March 24 fluid was accumulating in the right pleural cavity. That afternoon a fairly extensive pulmonary hemorrhage took place, succeeded by a large hemorrhage into the bowel. During the night, after a very severe paroxysm of coughing, the patient became extremely weak and could be roused only momentarily. Cheyne Stokes respirations began shortly thereafter. Copious emesis of dark red blood followed, after which the patient lapsed into deep coma, and death occurred at 7:40 A.M., March 25.

Clinical diagnosis. Acute aleukemic leukemia.

Table I shows blood counts which are of interest.

Autopsy Report, one hour postmortem (from the Department of Pathology, University of Wisconsin Medical School, Dr. C. H. Bunting, Director).

"*Gross Anatomical Diagnosis*—Generalized lymph node enlargement, hemorrhage into the subcutaneous tissue, mucous membranes, and serous surfaces, hemorrhagic bronchopneumonia, pulmonary infarction, splenic tumor with lymphoid hyperplasia, hydrothorax, left side, indeterminate vegetative endocarditis, aortic and mitral valves, cloudy swelling and fatty change in liver, transfusion incisions, right arm, abdominal scar of old appendectomy, pleural adhesions, right lung, chronic desquamative dermatitis, milk patch on heart, bicuspid aortic valve.

"*Microscopic Report—Lymph Nodes* (The same description will apply to all nodes examined, mesenteric, bronchial, superficial.) The architecture of the nodes is almost entirely obscured. A few small clumps of small lymphocytes are scattered through the gland sections, usually in the peripheral portion. The rest of the gland is infiltrated with cells chiefly much larger than the small lymphocytes. Many of these are of lymphoblastic character but usually with more extensive protoplasm. In many of these the nuclei are eccentric and lobed as in the monocyte. There are also many cells with extensive protoplasm and sharp vesicular nucleus with distinct nucleolus or nucleoli. Large cells in mitosis are numerous. Scattered among these large cells are intermediate sized and small lymphocytes.

"*Bone Marrow* Section of the bone marrow of the femur shows a diffuse infiltration with cells of the types described for the lymph nodes. Only scattered neutrophilic leukocytes, and an occasional small group of nucleated red cells are seen.

"*Heart* The epicardium shows a minute hemorrhage, and in certain areas a marked in-

TABLE I  
CASE 1

DATE	HB	R. B C	W B C	NEUTR.	LYMPH.		L M	MYEL	EOSIN	BASO	LYMPHO-BLASTS	NORMO-BLASTS	PLATE LETS	COAG TIME
					S	L								
1/27			9,100	85	14		1							
1/28			8,650	76	24									
1/30			7,800	91	8									
2/9			7,050	60	37				1					
2/13	79	4,830,000							3					
2/20			4,250	59	39		1		1					4 min
2/27														
3/6			3,900	15	81		1	1	2					
3/7	57	3,280,000	4,150	23	39				1		37	2		
3/8	59*	3,400,000	4,250	30	45				1		24	2	140,000	
3/9			3,950	25	43		1	1			30	1		
3/11	55	3,390,000	4,500	20	61				4		15	2		
3/12	62*	3,890,000	4,850	20	53	1		3			23	1		
3/15	60	3,490,000	5,600	5	58			1			36	1	70,000	
3/21	58	3,770,000	7,700	10	51	1	1				38	1		
3/22	59	3,270,000	10,000	7	21			1	1		70			
3/23	54	3,150,000	12,150	4	15						81	1		
3/24	54	3,150,000	15,500	2	28	1					69	1		

\*There are very obvious discrepancies in the classification of the various white cells from this point onward as routine blood examinations were not successful in identifying all the aberrant forms present in the smears. Dr. Bunting's description of mesenchyme cells showed wherein the error had existed so that these counts are of value only in pointing the general trend of the blood picture. It is probable that practically all of the cells classified as lymphoblasts were monocytes.

\*After transfusion

filtration of its adipose tissue with large mononuclear cells of the types described. Heart fibers in some bundles are definitely hypertrophied. Close to the endocardium one bundle is markedly atrophic. Around the arterioles there is a definite fibrillar increase in connective tissue, the fibrils forming somewhat concentric lamellae, suggestive of rheumatic scars. One cellular Aschoff body is found adjacent to one small vessel.

"*Lung* Section 1. Section shows congestion and edema, with some infiltration of the alveolar walls in the subpleural part with large cells of the character described.

"Section 2. Shows an area of dense hemorrhage with necrosis of lung framework, on its periphery in one area there are numerous groups of coccoid hematoxylin staining organisms with considerable leucocytic reaction. In general in the periphery there is a wide zone in which there is an alveolar exudate consisting of a fibrin network, enclosing red blood cells and numerous large and small mononuclear cells with only an occasional polymorphonuclear leucocyte.

"Section 3. Section shows edema, hemorrhage, organized pneumonia, and many areas of interstitial accumulation of leucemic cells. These are especially marked about blood vessels. Many phagocytes in alveoli contain fat vacuoles.

"*Spleen* Malpighian corpuscles are small. Pulp is increased in amount due to extensive accumulations of leucemic cells, of the character described.

"*Liver* Parenchymatous degeneration in atrophic liver cells. Sinusoids dilated and crowded with atypical mononuclear cells, numerous large cells in blood stream in mitosis. V. Kupffer cells prominent, phagocytic for red cells and lymphocytes and cell pigments.

"*Kidney* Convoluted tubular epithelium in general granular, much swollen, closing the tubular lumen. In certain tubules minute fat vacuoles are present in the cells. Glomeruli and blood vessels appear normal. Hemorrhage and infiltration with mononuclear cells in adipose tissue.

"*Pancreas, Gall Bladder, Urinary Bladder, and Prostate* Sections appear normal.

"*Aorta* Thin walled but with no definite lesion.

"In general vessels show leucemic clots.

*"Histologic Interpretation — Acute leucemia involving lymphoid apparatus and bone marrow, resulting in proliferation of atypical large mononuclear cells and of endothelial cells (primitive reticuloendothelium)*

*"Old and recent rheumatic lesions in heart Infected intarets in lung Bronchopneumonia Interstitial leucemic deposits Leucemic spleen"*

#### DISCUSSION OF CASE 1

There are several points of unusual interest suggested by this case The patient came under observation early and except for a four-day interval was con-

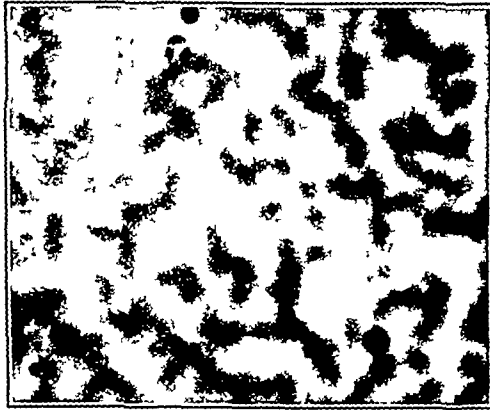


Fig 1 —Microphotograph of smear from Case 1 March 11, 1930 Magnification  $\times 600$  Field containing four cells (1) Large endothelial cell (2) polymorphonuclear neutrophile leucocyte (3) small lymphocyte (4) large lymphocyte or monocyctoid cell



Fig 2 —Microphotograph of smear from Case 1 March 11, 1930 Magnification  $\times 600$  Endothelial cell and polymorphonuclear neutrophile leucocyte

tinuously observed from the onset of symptoms until the fatal termination of the disease Many students with minor illness who under ordinary circumstances would not be hospitalized are, because of the exigencies of rooming house existence, admitted to the Student Infirmary for care This was such a case, at the onset Up to the time of the tonsillectomy the blood picture was essentially normal and there was nothing to suggest the true nature of the disease There are no characteristic early symptoms of acute leucemia Frequently vague pains such as this

patient complained of are reported. Sometimes the onset suggests acute rheumatic fever. No satisfactory explanation is offered to account for such pains. Possibly they are due to myositis, neuritis, or arthritis of toxic origin.

The question arises as to what influence if any the tonsillectomy had on the course of the disease, though it probably had none. As noted, the blood picture was essentially normal until the time of operation, but in retrospect we can see that the last white count previous to operation, 4,250 leucocytes, indicated a begin-

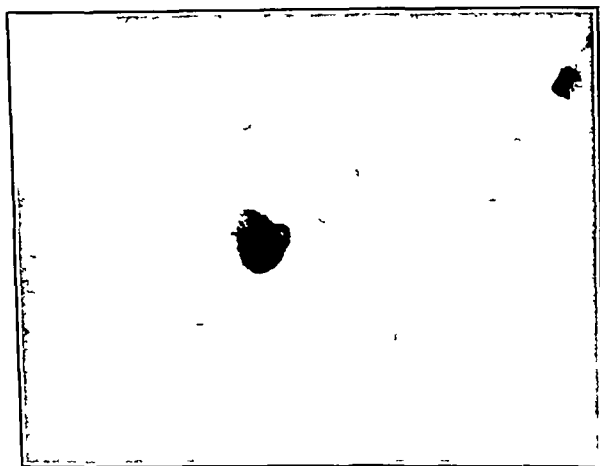


Fig. 3—Microphotograph of smear from Case 1 March 11, 1930 Magnification  $\times 600$  Endothelial cell

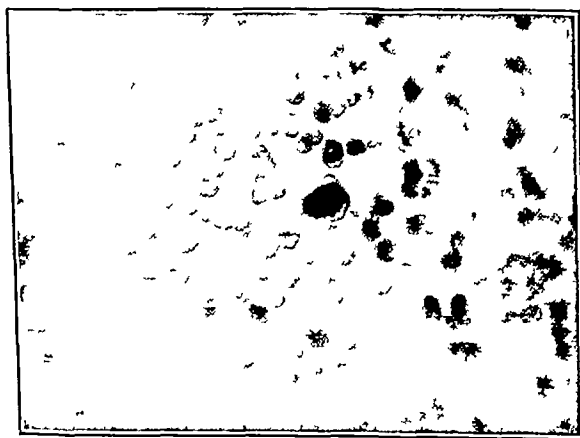


Fig. 4—Microphotograph of smear from Case 1 March 11, 1930 Magnification  $\times 600$  Endothelial cell

ning leucopenia which became more marked shortly after operation. Changes in the differential count, however, were not present until after operation. It is unfortunate that no blood count was made later than one week previous to operation. Of interest in comparison with the present case is one of "excessive hemorrhage after tooth extraction, due to acute leucemia," described by Walmesley.<sup>27</sup> A girl of seventeen bled continuously following tooth extraction until her death on the eighth day. Necropsy revealed acute lymphatic leucemia. An inquest was

held at which it was maintained that, "It was impossible to say that the extraction had accelerated death, and probably it was a coincidence."

The diagnosis of acute leucemia is obvious from the clinical course and the abnormal blood picture. The latter was aleucemic at first but the white count rose to 15,500 before death. The determination of the *type* of leucemia depends upon the identification of the abnormal cells in the blood stream and upon the necropsy findings.

The abnormal cells are not of the type ordinarily found in either lymphatic or myeloid leucemia. The routine counts classify them as "lymphoblasts," but according to Dr. Bunting's report they should be considered extremely primitive cells. The large numbers of small lymphocytes in the smears are in marked contrast to the usual findings in acute lymphatic leucemia, where the leucocytes are almost exclusively large lymphatic elements. The majority of the neutrophile leucocytes show nonsegmented nuclei, usually regarded as indicating immaturity, but transition stages are not seen between them and the very large leucemic cells. Besides the "striking cells" described by Dr. Bunting, there are many cells varying in size from the largest of those just mentioned down to about 14 by 16  $\mu$ , similar in appearance except that the protoplasm is more deeply basophile and completely filled with close-set granules instead of having a clear border. Many of these cells have nucleoli. Most of them are round or oval in shape but some are irregular in outline, and many are polygonal, very comparable in shape to ordinary squamous epithelial cells. (The latter may be distorted.) The smaller of these cells approach the morphology of ordinary monocytes. The absence from the routine counts of cells classed as large mononuclears indicates that entirely characteristic mature monocytes are rare. Sections of the spleen, liver, bone marrow, and lymph nodes show infiltration with cells similar to those found in the blood smears. (Figs 1-4 are microphotographs showing characteristic cells from a smear made March 11, 1930.)

Case 2—G. W., a white female, housewife, aged thirty four, was admitted to the Wisconsin General Hospital May 29, 1925, her chief complaint being pain in the arms. Two days prior to admission she had experienced pain in the hips and back which shifted to the arms a few hours later. This was severe enough to prevent sleep, and although at first relieved by aspirin was now apparently not amenable to such medication. The pains were sharp and shooting, changing to soreness after a few hours.

History showed that there had been transient stiffness and some pain in the arms for two years previously. Lately these limbs had felt stiff most of the time. Previous attacks had been well controlled by aspirin. She complained of a few dark discolorations on the skin, resembling bruises but without history of injury. There was a pain in the right chest resembling that in the arms, somewhat aggravated by deep breathing or coughing. History included chronic constipation, and some leucorrhea and menorrhagia.

Past medical history. Measles, chicken pox, whooping cough as a child. Mild influenza in 1920. Many attacks of tonsillitis. Two attacks of quinsy. Tonsillectomy in 1923. There had been two normal deliveries, no miscarriages, the last baby was nine months old at the time of the patient's admission.

Social history and family history. Irrelevant.

Physical examination showed gingivitis, slight inguinal adenopathy, ecchymotic areas on the limbs, cervical laceration and erosion, third degree uterine retroversion. The diagnosis was bilateral brachial neuritis, probably secondary to chronic endocervicitis, gingivitis. Additional history on June 16, 1925, told of mild attacks of nausea for years. There were sudden seizures



of nausea and cramplike pain in the epigastrium, usually coming on while the patient was eating. Symptoms were relieved by induced vomiting. Impression: Chronic cholecystitis, possibly with cholelithiasis.

X-rays of the paranasal sinuses and left shoulder were negative, of the teeth showed an impacted unerupted upper left third molar with slight periapical involvement of the upper right second bicuspid. Roentgenologic studies of the gastrointestinal tract showed nothing abnormal except a moderately delayed emptying time for the stomach due to hypomotility. Fractional gastric analysis was done and showed an achylia and the constant presence of occult blood. Other laboratory examinations included urine, containing traces of albumin and traces of glucose, a negative blood Wassermann, normal blood chemistry, normal coagulation time and fragility test, and blood counts within limits of ordinary observation.

Treatment consisted of local applications to the gums, and electric cauterization of the lacerated cervix together with pessary support of the retroverted uterus. She was discharged from hospital on June 19, 1925. Patient was readmitted to hospital one week later for further study, as it was felt that the original diagnosis had not been complete. She had gained three or four pounds in weight, but the painful symptoms had recurred.

Reexamination of the stomach and duodenum roentgenologically gave normal findings. Pelvic examination disclosed excellent healing in the cervix, and the pelvic condition generally was considered normal. Spontaneous slight bleeding from the gums continued, and smears showed presence of *Entameba gingivalis*. Fresh stools showed flagellates. There was now a prolonged bleeding time, and repeated blood counts showed a gradual dropping in the number of leucocytes as well as in the erythrocytes and hemoglobin. The most significant feature of the blood picture was a continued lowering of the percentage of granular leucocytes and the blood platelets. The temperature, which had been normal for the first four days, began to show daily variations between 99° and 103°, of a septic type.

The diagnosis now arrived at was that of a leucemia of the aleucemic type. The general condition became progressively worse until July 28, when the first transfusion was given with definite improvement for a period of several days. By August 23 pain was returning, being now present in the left side of the face, the chest, and the lower back and thighs. On September 5 chest examination showed marked elevation of the right diaphragm, thought to be due to palpable enlargement of the liver, confirmed by x-ray. Transfusions of citrated blood were given on three subsequent occasions, the first two resulting in temporary improvement, the third being apparently without effect. Horse serum was also given, with temporary benefit. Marked cervical and inguinal adenopathy had been present as early as September 30. From October 21 the patient declined very rapidly. Pain in various areas became steadily worse, and bleeding from the gums increased. There was considerable vomiting. Semiconsciousness supervened by October 25 and the patient died early the following morning.

Autopsy Report, one hour postmortem (from the Department of Pathology, University of Wisconsin Medical School, Dr. C. H. Bunting, Director).

*Gross Anatomical Diagnosis*—Lymphocytic leucemia, exhaustion of bone marrow, anemia, fatty degeneration of parenchymatous organs, zonal necrosis of liver, splenic tumor, auricular thrombus, embolism of pulmonary artery, infected intaret right lung, pneumonia, cholelithiasis.

*Microscopic Report—Lymph Nodes* The general architecture of gland has disappeared. Small groups of lymphocytes suggesting the Malpighian corpuscles of the spleen are grouped about the arterioles in the region of the former germinal centers. The rest of the gland is diffusely infiltrated with large pale cells. These vary in size and character. Some evidently belong in the primitive stages of the lymphoid series, while many are definitely of an endothelial character with abundant protoplasm and large vesicular nucleus with definite nucleolus. Mitotic figures among these cells are numerous.

*Spleen* The Malpighian corpuscles are small, consisting in the main of small lymphoid cells. The pulp of the spleen and its sinuses are crowded with cells of the character of those described for the lymph nodes. Considerable hemosiderin is found in phagocytes in the pulp.

*Rib Marrow* The rib marrow shows almost complete exhaustion of hemopoietic centers, and atrophy of fat. The vascular architecture is well marked, also the reticular structure of

TABLE II\*  
CASE 2

DATE	RED BLOOD CELLS	WHITE BLOOD CELLS	NEUTR LYMPH	S LYMPH	L LYMPH	INT	L M	MYE	TRANS	POSI	RASO	LYM BLO BLASTS	NORMO BLASTS	UNNAMED MONO CYTOLS (PATH)	DEGEN CHANGES IN R. B. C.	PLAQUETS	COAG TIME	DIFF TIME
5/30/82	4,430,000	9,000	56.5	29	26		10.5		0.4	0.7	0.3						7 1/2 min	
6/8/76	4,430,000	6,750	58	37	2				2	1								
6/18/82	4,370,000	4,900	49	38	9				4									
6/30/55	4,055,000	3,500	39.2	41	17		2			0.4	0.4							
7/10/55	3,280,000	3,600	46.2	32.8	46		1.2	1	0.2	1.6								
7/29/43	2,960,000	1,300	26.2	53.6	6		4.2	1		2								25 min
8/17/50	2,610,000	3,850																7 min
8/26/53	3,660,000	4,200																2 1/2 min
9/14/40	3,350,000	5,900	18.6	71.8	53													7 min
9/28/20	2,065,000	9,850	9	60.5	30.5					0.2	0.2							17 min
10/6/23	1,940,000	15,650	3.8	25	26		44.6	0.2										62 min
10/15/15	1,492,000	20,600	2.2	25.4	15.8		48.6											45 min

\*As in the first case these blood counts are to be regarded only as a rough indication of the general blood picture. Smears of the blood were examined by Dr. C. H. Bunline and found to contain large atypical cells of the same type described by him in Case 1.

the marrow There is some edema, areas of hemorrhage with fibroblastic proliferation, also a few perivascular collections of large cells of the type described There are also scattered lymphocytes in the marrow

*"Femoral Marrow"* Similar to rib marrow, with possibly larger perivascular collections or primitive cells

*"Heart"* Section shows congestion and edema of heart wall, slight atrophy of muscle fibers, with hydropic degeneration

*"Heart Thrombus"* Shows dense almost hyaline platelet columns, with heavy fibrin border and well marked intervening fibrin network, containing chiefly red blood cells and atypical leucocyte forms The usual neutrophile border to the platelet columns lacking

*"Lung"* Small area of necrosis of lung tissue with many colonies of gram staining cocci in it Surrounding areas of pneumonia, hemorrhage, and edema Pneumonic exudate shows well marked fibrinous network with a high proportion of neutrophile leucocytes in some areas, and in others chiefly the large mononuclear type of cell

*"Liver"* Sinuses of liver lobule dilated and containing many mononuclear leucocytes General atrophy of liver cells of central and mid zone of lobule, with fatty degeneration and some necrosis Cells of peripheral zone are somewhat swollen, granular

*"Kidney"* Tubules of cortex much dilated and containing heavy albuminous precipitate Cells of tubules swollen, granular and hydropic Capsules of glomeruli are dilated, albuminous precipitate In the boundary zone and medulla the interstitial tissue shows numerous collections of cells of great variety There are megalokaryocytes, neutrophile and eosinophile leucocytes and myelocytes, and nucleated red cells Suggesting a myeloid transformation

*"Pancreas"* Atrophy, lipomatosis, well marked interacinar pancreatitis, islands generally, hypertrophic

*"Aorta"* Atrophy and degeneration of media Atheromatous nodule in intima

*"Uterus and Ovaries"* Senile atrophy "

#### DISCUSSION OF CASE 2

There are several points of similarity with Case 1 This patient also was observed from the onset of symptoms until the fatal termination There was an onset with vague pains leading to a diagnosis of neuritis, and a focus of infection was considered to be the etiologic factor At first there was nothing to suggest the true nature of the case, except the presence of gingivitis and ecchymotic areas The blood counts during the first admission were essentially normal Here, as in the first case, there was gradual decrease in the granular leucocytes with increase in cells classified as lymphocytes The total number of leucocytes dropped to a low level of 1,300 per c mm but toward the end rose to 20,600 The course of the disease was somewhat less acute than in the first case, extending over a period of five months as contrasted with two months in the former Here, as in the first case, a typical clinical and blood picture of leucemia gradually developed While the gross anatomical diagnosis was lymphocytic leucemia, microscopic examination showed the type cells to be very primitive in character Some of them evidently belonged in the primitive stages of the lymphoid series but many were definitely of an "endothelial" character

#### GENERAL DISCUSSION

These cases of leucemia are presented for their clinical interest in that both were followed from the onset of symptoms to a fatal termination and necropsy, and also because of the very unusual blood pictures Although, unfortunately, the clinical study throws no light on the etiology of this disease, nor the pathologic study on the origin of the abnormal blood cells, it is hoped that the addition to the

literature of two more cases of an unusual type of leucemia may be of some value as an addition to the data available for study

The exact classification of these cases in relation to others previously published is difficult. They appear to differ somewhat from the cases reviewed by Dameshek,<sup>7</sup> and the more recent cases of monocytic leucemia in the absence from the blood stream of large numbers of typical monocytes. They perhaps are allied to the rather heterogeneous group of primitive cell leucemias described under various names by Bykova,<sup>4</sup> Feller and Risak,<sup>9</sup> Hittman,<sup>12-13</sup> Hoff,<sup>14</sup> and others.

Most of the reports of monocytic leucemia have favored a reticuloendothelial origin for the monocyte and several authors have described transitions from reticuloendothelial cells through free histocytes to mature monocytes. In using the term monocytic leucemia here, there is no intent to advocate any particular theory of origin of the characteristic abnormal cells or of monocytes in general.

A review of the varied hypotheses gleaned from a review of the literature on monocytic leucemia and the monocytes may be of interest.

There seems to be general agreement among hematologists that in postnatal life undifferentiated mesenchymal cells exist which may under certain circumstances give rise to all forms of blood cells. By the polyphyletists these are considered to be the only pluripotent "stem cells." The monophyletists believe in a hemocytoblast, a derivative of the primitive mesenchyme, which *normally* gives rise to all forms of blood cells. It would appear that there is considerable confusion of terms and ideas relative to the stem cells. By many writers the reticuloendothelial system is regarded as synonymous with primitive mesenchyme. Maximow,<sup>16</sup> on the contrary, ascribes very limited blood forming powers to the reticuloendothelial system and describes other "undifferentiated mesenchymal cells." He says, "In the reticulum of the lymphoid tissue of vitally stained animals, the large phagocytic reticular cells which store the dye, the typical histocytes, can be distinguished from small, pale elements of the syncytium, which contains few or no inclusions and are especially distinct in the germ centers." He claims that these syncytial elements, found in the common connective tissue as well as in that of the blood forming organs, are "fixed undifferentiated cells which keep their embryonic mesenchymal potencies."

To illustrate the confusion of terms and the lack of distinction between reticuloendothelial cells and undifferentiated mesenchyme the following may be cited. Pinkerton<sup>20</sup> uses synonymously the terms "adventitial cells," "clasmatocytes," "polyblasts," and "hematohistoblasts," and considers them "*undifferentiated mesenchymal elements*." Dameshek<sup>7</sup> names as constituting the "*reticuloendothelial system*" cells variously called "clasmatocytes," "macrophages," "resting-wandering cells," and "histocytes." He later uses as synonymous "hemocytoblast," "hemohistoblast," "histocyte," and "Stammzell." Richter<sup>22</sup> says of the hemohistoblast of Ferrata, "Morphologically the cell has been identified with the group of tissue cells variously known as 'clasmatocytes' (Ranvier), 'resting-wandering cells' (Maximow), 'adventitial cells' (Marchand), '*reticuloendothelial system*' (Aschoff)." He agrees with Ferrata in considering the hemohistoblasts "*very element mesenchymatici embryali*." Maximow<sup>16</sup> considers "lymphocyte" and "hemocytoblast" synonymous. He says, "The so called unitarian theory—

claims that there is only one stem cell and that the common small lymphocyte of the blood "

Numerous cases called, from their pathologic picture, leucemic reticuloendothelioses have appeared in the literature during the present century. Many of these have shown in the blood stream, in addition to histocytes, large numbers of cells described as typical monocytes. By those believing in the reticuloendothelial origin of the monocyte it is felt that the term leucemic reticuloendotheliosis should be applied to all monocytic leucemias. The term "histocyte" is used with such a variety of connotations that it leads to some confusion. At times it is used as synonymous with reticuloendothelium, again it serves to designate only *free* reticuloendothelial elements either in the blood stream or in the tissues. By many the term is used to mean those mononucleated cells in the blood stream which they regard as immature monocytes, transitional forms between fixed reticuloendothelial cells and mature monocytes. The histocyte is thought by them to bear the same relation to the monocyte as the myelocyte to the mature leucocyte. Dameshek's<sup>6</sup> recent paper on the histocyte mentions this point of view. The essential rôle of the reticuloendothelium in leucemias in general is discussed by Bykova<sup>4</sup> in a recent article (1931). He quotes from Hoff, "The reticuloendothelial system under pathologic conditions can differentiate in various directions according to the type of stimulus and lead to myeloid, lymphoid, or monocytoid leucemias and their combinations." Two cases of Hittmair (V and VI)<sup>12-13</sup> serve to illustrate this fact. In Case VI at one period myeloid cells predominated, at another, lymphatic, and at a third period, more primitive cells. Case V was a chronic case treated by x-ray which showed ten exacerbations. At one time "lymphoidocytes" dominated the picture, at another reticuloendothelial monocytes, and again cells of the myeloid series. The case of Wyschegorodzewa<sup>29</sup> cited by Dameshek as one of monocytic leukemia showed at the end a change from a monocytic to a myeloid character. The author comments that this change speaks for the possibility of formation of myeloid elements from monocytes. It is conceivable, however, that early in the disease the reticuloendothelium was stimulated to the production of monocytes and toward the end, for some reason, to the production of myeloid cells.

The question of the origin of the monocyte is still much debated. Besides the theory of direct reticuloendothelial origin, Bloom<sup>1</sup> cites other theories. (1) origin from myeloid cells, (2) origin from lymphatic cells, and (3) origin from ordinary vascular endothelium. Cunningham, Sabin, and Doan<sup>5</sup> suggest an origin from a "primitive blood cell" derived in turn from a reticulum cell. Early writers did not distinguish between monocytes and free histocytes. Sabin, Doan, and Cunningham<sup>23</sup> in 1925 distinguished in the tissues by the supravital technique two types of phagocytic cells which they called elasmatoocytes (the histocytes of other authors) and monocytes. There seems to be very general agreement that the histocytes (elasmatoocytes) are probably direct derivatives of reticuloendothelium. In fact, one frequently finds the terms reticuloendothelial system and histocytic system used interchangeably. Views as to the origin of the other large mononuclear phagocytes usually referred to as monocytes remain divided as indicated above.

The reasoning in practically all instances is that, under various circumstances, all transitional stages can be found between the monocyte and the cell from which the particular observer believes it to be derived. We may cite, e. g., the following observations in favor of the several theories.

*Myeloid Origin* Pincus<sup>19</sup> has seen all transitions between myeloblasts and monocytes. He describes a "promonocyte" which is sometimes impossible of differentiation from a myeloblast.

Ehrlich believed he saw transitions from large mononuclears, by way of the transitional cells, to neutrophile granular leucocytes. (This observation is now generally conceded to be incorrect. Nagels, the chief follower of Ehrlich in supporting the myeloid origin of the monocytes, bases his opinion on the *oxydase reaction*.)

*Lymphatic Origin* Weidenrich and others (according to Bloom<sup>1</sup>) have described a morphologic similarity between lymphocytes and monocytes with transitional stages.

Bloom, himself, using various stains including the supravital, saw in smears of the blood, and in smears and sections of the blood forming organs, all transitions from lymphocytes to monocytes. His observations were made on animals in which mononucleosis had been induced by injections of B mononucleogenes or colloidal substances.

"*Primitive Cell Origin*" Sabin, Dorn, and Cunningham<sup>2</sup> state that splenic puncture shows all steps in the origin of the monocyte from the primitive blood cell. They have seen, also, in tuberculous organs very young monocytes touching a primitive cell on the one hand, and mature monocytes on the other.

*Histiocytic Origin* Schilling believes (Bloom<sup>1</sup>) that in endocarditis lenta and monocytic leucemia he has seen transitions between histiocytes and monocytes.

Swirschewskaja<sup>3</sup> describes a case of "reticuloendotheliosis" in which the blood contained typical monocytes, and in which after necropsy, sections of the spleen showed all transitions from the endothelial cells lining the splenic sinuses to free cells of the monocyte type.

Dameshek<sup>7</sup> notes that a direct association between proliferative reticuloendothelial cells and the leucemic cells has been described in monocytic leucemia by several authors.

It would seem either that the monocytes have a multiple origin or that the method of determining origin of a cell by morphologic similarity is unreliable.

A new method of attack was used by Silberberg<sup>25</sup> which seems definitely to disprove an exclusive myelocytic origin of the monocytes. By poisoning with benzol he obtained animals in which the circulating blood was free of granular cells. Monocytes were still present in the circulation. Tissue cultures of the spleen and bone marrow from these animals showed histiocytes and monocytes with all transitions, as well as lymphocytes but no cells of the myeloid series. No mention is made of transitions from lymphocytes to monocytes, so that this work favors the histiocytic origin of the monocyte as opposed to the lymphatic, although it does not definitely exclude the latter. In cultures of spleen and bone marrow from leucocyte-free animals vitally stained with carmine, he saw some carmine in the monocytes as well as in the histiocytes. One of Bloom's<sup>1</sup> arguments against the histiocytic theory was that none of the monocytes in his vitally stained animals took the vital dye. A possible weakness in these experiments is the identification of monocytes by the supravital stain. Hall<sup>21</sup> has shown in his review of the literature on supravital staining that this method cannot be relied upon for the identification of monocytes "because there are too many other types of rosette cells in the tissues that may be confused with monocytes."

The oxydase reaction, usually considered a criterion of myeloid cells, is apparently of little aid in the solution of the monocyte question. Monocytes generally are described as oxydase positive, but Ordway and Gorham<sup>18</sup> in the *Oxford Monographs* state that "a small percentage, perhaps 5 per cent react negatively." Schilling<sup>24</sup> says they are either negative or faintly positive to the oxydase stain. Dameshek<sup>7</sup> states that the oxydase reaction is faintly positive. Uyeyonahara (1930), according to Hall,<sup>21</sup> supports the theory of multiple origin of the monocytes. He reports a classification of hematogenous monocytes on a basis of peroxy-

dase reaction and storage of carmine colloidal dye. Monocytes showing either a negative or positive peroxidase reaction and containing carmine granules are derived from histocytes, those possessing peroxidase granules but no carmine granules are of myeloid origin, and those containing neither carmine nor peroxidase granules are of lymphatic origin. His method consists of incubating citrated human blood at body temperature and exposing it for varying intervals of time to a solution of carmine. Dry smear preparations are made and stained for peroxidase granules. Hall<sup>11</sup> considers this classification questionable, for Downey offers convincing evidence to show that dye storage cannot be used as a criterion for separation of the various cell elements in the blood and tissues. Bock and Wiede<sup>2</sup> feel that it is likely that even yet diverse forms are grouped together under the name of monocytes.

## SUMMARY

Two cases of leucemia, duration two months and five months respectively, are reported. Certain similarities of early symptomatology are noted, as well as corresponding difficulty of recognition of aberrant cells in the blood smears. In each instance, the predominating cell was too primitive to admit of hard and fast classification. Other cases, apparently parallel in most respects, are mentioned. A brief review of the literature concerned with the rôle of the reticuloendothelium in leucemia is included, together with the views of various authors on the origin of the monocyte.

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# LABORATORY METHODS

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## FURTHER STUDIES ON A COMPARISON OF THE HUDDLESON SLIDE TEST WITH THE MACROSCOPIC TUBE TEST IN UNDULANT FEVER\*

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IN A previous article<sup>1</sup> the authors demonstrated that the Huddleson "Rapid Method" for determining the presence of agglutinins of the *Brucella* group in the blood sera of human beings was slightly more sensitive and specific than the macroscopic tube test used. The rapidity and ease with which the Huddleson test may be carried out made it an excellent test for public health laboratories where speed and accuracy in reporting results to physicians are important factors.

Since our previously published series included only 156 specimens it seemed desirable to continue the investigation. For the past year a total of 763 examinations for the presence of agglutinins for the *Brucella* group have been made.

### METHODS

The first 120 sera were examined by three methods, two macroscopic tube tests and the Huddleson method. One of the tube tests was done for us by Dr W N Plastridge at the Connecticut (Storrs) Agricultural Experiment Station and the method has been reported previously.<sup>1</sup> The Huddleson "Rapid Method" has been described in a number of earlier publications.<sup>2 3 4</sup> The third test—a macroscopic tube test—is described below as the "Conn" test.

The antigen employed in the Conn test noted above was made from four strains of *Brucella abortus*, coming from different sections of the country, whose agglutinability had been carefully checked. The strains were grown for twenty-four hours on 1 per cent glycerine Fairchild's agar and transplants were made from these onto plain Fairchild's agar and incubated forty-eight hours at 37° C. The growth was harvested with a small amount of physiologic salt solution containing 0.4 per cent phenol and diluted for test purposes to a reading of 7.8 to 8 on the Gates' apparatus.

Test tubes, 75 by 10 mm, were used, 0.5 c.c. of diluted serum being placed in each tube, the dilutions ranging from 1:25 to 1:3000 in a regular sequence and 0.5 c.c. of antigen finally being added to each. The tests were incubated in a 56° C water-bath for six hours followed by overnight in the refrigerator at 8° C. The degree of clumping was estimated and tabulated at +, ++, +++ or ++++ representing respectively 25 per cent, 50 per cent, 75 per cent and 100 per cent agglutination.

### RESULTS

An arbitrary classification has been in use for several years for the routine reporting of results

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Specimens showing titers of 2-, 3-, or 4+ in any dilution or dilutions including 1:300 and above are classed as "positive." Specimens are classified as "questionable" if they show any reaction either above or below 1:300 when they fail to fall in the positive class. All other examinations are reported as negative. This standard was purely arbitrary but gave us a definite basis for the reporting of specimens.

As previously reported,<sup>1</sup> in our experience active clinical cases of undulant fever rarely give a positive agglutination reaction that is not above a titer of 1:100 although a positive reaction in a titer of 1:100 is considered diagnostic by some.

The results obtained from all three tests on 120 sera are given in Table I.

TABLE I  
RESULTS OF AGGLUTINATION BY THREE METHODS ON 120 SERA

METHOD	POSITIVES	QUESTIONABLES	NEGATIVES	TOTALS
*Storrs	10	30	80	120
Huddleson	9	9	102	120
*Conn	9	5	106	120

\*Macroscopic tube method

It is at once apparent there is close agreement as to "positives" by the three methods. The Storrs method on the basis of our arbitrary classification gave 10 positives as against 9 by the other two methods. The physician in charge of the case found positive by the Storrs method but questionable by the other two methods, stated that the patient's history failed to show symptoms of undulant fever.

The questionable reactions obtained were considerably greater with the Storrs method than with the other two (note Table I), the former method giving 30 questionable results as against 5 and 9 respectively for the Conn and Huddleson methods.

Table II gives the reactions obtained with 763 serum examinations for the year 1931 with the Conn and Huddleson Rapid methods.

It will be noted that the Conn test (macroscopic tube) gave six more positives than the Huddleson on the basis of our standards for laboratory diagnosis. In each of these six serums, however, the Huddleson did show some reaction at least in the 1:100 dilution which, as has been pointed out, is considered diagnostic by some laboratories. Further, two of these serums were repeated tests which previously had shown an agglutination in the 1:3000 dilution with the Huddleson method. Further, it will be noted (Table II) that the tube test showed 45 more questionable reactions than did the Huddleson method, indicating a definite difference in specificity of the two tests.

In order to obtain more accurately the value of the tests used for the diagnosis of undulant fever, questionnaires were sent to all physicians with laboratory-diagnosed positive cases. In Table III these results are summarized. It will be

TABLE II  
RESULTS OF AGGLUTINATION WITH CONN AND HIDDLESON METHODS ON 763 SPECIMENS

METHOD	POSITIVES	NEGATIVES	QUESTIONABLES	TOTALS
Huddleson	*59	645	*59	763
Conn	65	594	104	763

\*Note. There were eleven repeated tests. In two of these the Huddleson had dropped from a titer of 2000 to 100 and hence fall in questionable group.

noted that there were 31 cases of undulant fever so diagnosed by physicians which gave positive agglutinin reactions. There were eleven tests repeated in the series of 65 laboratory positive cases, 22 positive by agglutination reported negative by physicians and one case undiagnosed. In this undiagnosed case and in those cases numbered 47,467 through 69,261, although a diagnosis of undulant fever was not made in most instances, definite fever symptoms developed during the illness. In specimen 56,846, in which no diagnosis was made, agglutinins for the *Brucella* group were present in the 1:3000 dilution and the clinical evidence indicated fever symptoms suggestive of typhoid, yet there were no agglutinins for *Eberthella typhi* nor did rose spots develop. In one case listed in this group of undiagnosed cases, 46,532, the patient was said to present no symptoms of any disease and appeared well, yet agglutinins for the *Brucella* group were present in a dilution of 1:3000. Such high titered agglutinins seem to us necessarily to indicate a *Brucella* infection in spite of the absence of classical clinical symptoms. It will be noted in Table III that the age group in which the diagnosis was most often given definitely as undulant fever was in the adult group, whereas cases in children and young adults were more often called either mild, atypical, or not undulant fever.

Specimen 69,261 (child, aged three and one-half years, Table III) was not clinically diagnosed as undulant fever. The titer by the Huddleson method was 1:100 and by the macroscopic 1:300. This was reported as a positive case by these Laboratories inasmuch as it fell within the range of our arbitrary standards. The physician reported an unexplained fever "Fever 99° to 100.8° for several weeks with a moderate pharyngitis and very slight cervical adenitis as the only physical signs." The agglutinin titers were comparatively low in this particular case but seem to us sufficiently high to raise the question whether or not a mild *Brucella* infection did not exist. In addition, the physician notes that this child had drunk considerable quantities of raw milk.

Sasano<sup>6</sup> and his co-workers report that "the examination of serums from 1000 persons revealed a positive complement fixation for *Brucella abortus* in 96 and a positive agglutination in 78. In only five cases was the diagnosis of undulant fever made. In each of these five cases complement fixation was positive, and agglutination was present in titers of from 1:135 to 1:200 at some time during the illness." Sasano further notes that a diagnosis of undulant fever should not be made on the basis of agglutinations in dilutions of 1:15 or 1:45 in the absence of a positive blood culture. A study of the case mentioned in the preceding paragraph and others to be discussed later indicates that extremely mild cases of

TABLE III

ANALYSIS OF SIXTY FIVE LABORATORY POSITIVE CASES OF BRUCELLA INFECTION

SPECIMEN NUMBER	PHYSICIAN'S DIAGNOSIS	LABORATORY TESTS		PATIENT'S AGE	REMARKS
		HUDDLESON	MACROSCOPIC		
85080	U F	3000	3000	None given	
11306	U F	2000	300	29	
10879	U F	3000	1500	31	
11530	U F	3000	3000	23	
521	U F	3000	3000	None given	
73733	U F	3000	3000	45	
3643	U F	3000	3000	46	
88241	U F	3000	3000	32	
35593	U F	3000	3000	35	
59748	U F	3000	1500	None given	
42176	U F	3000	1000	57	
35944	*U F	3000	3000	57	Repeat on 42476
5115	U F	3000	3000	37	
6682	U F	3000	3000	32	
27287	U F	300	300	45	
39596	U F	3000	3000	None given	
71892	U F	3000	3000	60	
7365	U F	3000	1500	17	
64440	U F	1000	750	14	
77986	U F	3000	3000	43	
7800	U F	3000	300	29	
84827	*U F	3000	1500	29	Repeat on 7800
76557	*U F	100	200	29	Repeat on 7800
26918	U F	3000	3000	20	
37488	U F	3000	1500	45	
32510	*U F	1000	750	45	Repeat on 37488
30782	U F	3000	3000	35	
13831	U F	3000	1000	21	
47467	-	3000	3000	45	Fever symptoms
48374	-	3000	3000	16	Fever symptoms
44788	-	3000	3000	16	Fever symptoms
15698	-	750	300	None given	Habitual abortion
74696	-	3000	750	61	Fever symptoms
50240	-	3000	3000	None given	Diagnosis uncertain, moderate fever
46532	-	3000	3000	61	Patient well, no symptoms
56846	None made	3000	3000	62	Fever symptoms, suggestive of typhoid
69261	-	100	300	3 1/2	Unexplained fever
79769	U F	3000	2000	None given	
6686	U F	3000	3000	18	
27291	*U F	100	300	18	Repeat 6686 (two months later)
1685	U F	3000	1500	14	
6854	U F	3000	3000	16	Mild case
6856	U F	100	750	14	Mild case
21617	U F	2000	300	17	Mild case
75066	U F	3000	300	14	Mild case
10117	*U F	3000	1500	14	Repeat on 75066
22700	-	750	1000	14	N S No illness
25668	-	1500	750	14	N S No illness
6469	-	3000	1500	17	N S Coryza and fever (recovered)
22698	-	1500	1000	18	N S Bronchitis

Note U F = Undulant fever

\* = Repeat tests

N S = No symptoms of undulant fever A diagnosis of undulant fever was not made

TABLE III (CONTINUED)

SPECIMEN NUMBER	PHYSICIAN'S DIAGNOSIS	LABORATORY TESTS		PATIENT'S AGE	REMARKS
		HUDDLESON	MICROSCOPIC		
25666	-	3000	300	16	N S No illness
27289	-	1500	300	14	N S No illness
22710	-	2000	2000	18	N S After chickenpox
2191	-	3000	3000	17	N S Six tests on patient 2191
21874	*-	3000	2000		over a period of ten months
28226	*-	1000	1000		Physician states at no time
42279	*-	3000	3000		were symptoms of undulant
66667	*-	1000	750		fever present Upper respir
76022	*-	2000	500		atory infection and mumps
					during year
6684	-	50	300	19	N S Upper respiratory infec
					tion (3 days)
25242	-	Insufficient	500	15	N S No illness
31392	-	Insufficient	300	17	N S Upper respiratory infec
					tion (2 days) mild otitis
					media
2494	-	25	500	18	N S Upper respiratory infec
					tion—fever—coryza
26914	-	100	200	17	N S No illness previous to
					test
27572	-	100	500	15	N S Upper respiratory infec
					tion (mild)

undulant fever may exist and because of the vagueness of the symptoms not be recognized as such Borman notes (personal communication) a case which would not have been recognized except for a continued series of daily temperatures He reports as follows

"Patient, a boy aged nine years, had been suffering from a middle ear infection of undetermined etiology After subsidence of symptoms his mother, a trained nurse, continued to take his temperature daily It was noted that his temperature with occasional intermissions ran consistently above normal ( $\frac{1}{2}^{\circ}$  to  $1^{\circ}$ ), especially after hard play Boy fatigued easily No other symptoms The abnormal temperature was checked several times by the physician's thermometer and the mother's thermometer was checked by taking the temperature of normal individuals This condition remained for the better part of a year The ear condition was pronounced completely cured by two physicians Physical examination was negative for any abnormalities to which this condition might be ascribed X ray findings were negative for tuberculosis Urine was repeatedly normal

"A macroscopic agglutination test for undulant fever was made which was positive (++++) in 1:2560 dilution Based on this, a diagnosis of undulant fever, mild in nature, was made by the physician

"History of continued ingestion of raw milk

"When last heard of, one year after making test, boy was developing normally but continued to run slight temperature at intermittent periods "

Cases such as the above would be missed entirely under ordinary conditions \* The rise in temperature was slight and could be ascribed to the middle-ear in-

\*Since this investigation was completed a similar case was encountered  
Case P—Boy aged nine History somewhat like that of grippe Afternoon temperature 100.103 morning normal to 100 Physical examination negative except for loss of weight No pain No glandular involvement Only symptoms apparent temperature and lassitude White cell count 10,000 Widal Weil Felix Kahn and Wassermann tests negative The Huddleson rapid method showed complete agglutination in the 1:6900 dilution

fection during the illness. These cases indicate to us that very mild cases of undulant fever may exist particularly in children and unless followed closely would not exemplify themselves in observable symptoms.

Twenty-seven specimens (6,686 through 27,572) were received from one physician\* who was investigating an outbreak of undulant fever in one of the preparatory schools for boys in the State. Inasmuch as he was in charge of this group of boys he had an excellent opportunity to study the series of cases that developed. He has kept careful case records of the patients that showed both the typical and atypical types of undulant fever. On the group of specimens reported positive by these Laboratories and in which the patients showed no clinical symptoms of undulant fever, he has taken temperatures carefully over a period of weeks and recorded detailed case histories. A large number of specimens have been checked in other laboratories and the physician has checked in his own laboratory the agglutinin titers particularly in cases where clinical symptoms were not comparable to the serologic results. Only in one case was a definite discrepancy noted and in that instance it appeared to be due to a laboratory error.

Following the development of two cases of undulant fever (6,686 and 1,685), an investigation of the condition of the herd from which the milk was obtained was made by the physician. He found that one of the cows in the herd had aborted November 13, 1930 and since raw milk was being used from the herd he was suspicious that an outbreak was imminent. Several cases of abortion had occurred in the herd in 1926 and 1927 but no cases of undulant fever had developed in the school. The first case of recognized undulant fever appeared on November 26, 1930, about two weeks after the cow had aborted, and the second case (brother of the first) developed while the boys were on Christmas vacation. In the first of these cases the organism was isolated from both blood and feces. Although a number of attempts have been made to isolate organisms from other suspected cases, they have been unsuccessful.

A specimen of blood from each of the cows (about 100) was taken and examined by the physician for agglutinins for the *Brucella* group. Four were found positive. One was from the cow that had recently aborted. These four cows were disposed of and pasteurization of the milk started. No new cases of abortion have appeared in the herd since.

Shortly after the Christmas vacation, specimens of blood were taken from each of the boys and tests made for presence of agglutinins for the *Brucella* group. Specimens that were found positive or suspicious were sent to these Laboratories for further examination. About 240 boys in the school and about 60 employees were tested. The 20 cases reported positive by these Laboratories are included in the 27 specimens, 6,686 through 27,572 (Table III).

Specimens 6,686 (27,291 repeat) and 1,685 (organism isolated) were definitely considered by the physician as cases of undulant fever. Specimens 6,854, 6,856, 21,617, and 75,066 he called mild cases since symptoms, although slight could be considered as indicative of undulant fever. These six cases and one other not reported were the only ones in the group of twenty reported positive

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\*The authors wish to express their appreciation for the excellent cooperation of this physician. His name is withheld to preserve the identity of the school.

by these Laboratories that were considered by the physician to be indicative of undulant fever.

The 19 specimens, 22,700 through 27,572 were all considered clinically negative. There were no symptoms suggesting undulant fever and, although in some instances upper respiratory symptoms developed, they were usually mild. In six instances there was no illness whatsoever.

The six specimens numbered 2,191 through 76,022 present an interesting case in which, although no symptoms of undulant fever were demonstrable, a definite increase and finally decrease in titer was shown over a period of ten months. The physician has made a number of agglutination tests on this patient. The original examination was routine and only a slight titer was demonstrated. In following the titer in this case over a period of ten months there was demonstrated an increase to a titer of 6000 which finally decreased to 1:120. At no time did the physician note symptoms of undulant fever although the patient did have an upper respiratory infection and mumps during the year.

Following is the case report supplied by the physician:

"Case 26—Aged eighteen years. During a small epidemic of upper respiratory infection, in May, 1930, this boy was admitted to the infirmary with the first illness which he had had in many years. It was characterized by headache, nasal discharge, and fever (39.6°), but was quite mild as his temperature dropped to normal on his second day, and remained normal until his discharge three days later. Because he happened to be one of a group of persons on whom *Brucella* agglutination tests were being made in an effort to determine the incidence of such agglutinins among normal persons, his serum was tested at this time. No agglutinins were found. In July, 1930, he had a mild and uncomplicated case of mumps. On January 12, 1931, another routine serum test was made which was positive in 1:2560 dilution. On February 10, 1931, his serum was positive in 1:5120 dilution. This boy had been perfectly well since his cold in May, except for the mumps. He had no complaint whatever, and no sign or symptom of undulant fever, or of any other illness. A weight chart, recording monthly weighings, had been showing an uninterrupted gain in weight over a two year period. As soon as the positive agglutination was discovered, temperatures were taken twice daily for two weeks. Then an occasional evening temperature was taken over a period of five months, but, at no time, did he have any elevation above the normal. His stools were repeatedly negative for *Brucella* organisms, and so was his urine. During the winter, this boy carried a heavy program of studies successfully, and did a great deal of manual labor out of doors. He gained six pounds in weight despite the fact that he was somewhat overweight before. His hemoglobin remained at 95 per cent (Sahli). Apparently the only response which this boy made to his *Brucella* infection was his production of agglutinins. Nearly a year after the presence of agglutinins was first noted, his serum agglutinated *Brucella* in dilutions up to 1:120."

In some of these specimens, notably 2,494, 6,684, 25,242, 26,914, and 31,392 the agglutinin titers were comparatively low, yet all were well over the arbitrary lower limit of positivity. The possibility that these agglutinations are nonspecific seems to us to be slight, and certainly the reactions on the specimens whose titers run from 750 to 3000 can hardly be considered nonspecific.

#### DISCUSSION

The value of a diagnostic test in public health laboratories depends not alone upon accuracy in diagnosis but also upon the speed with which the test can be completed. In our former article on a shorter series of specimens, we found the Huddleson "Rapid Method" met these requirements. After further study

over a period of twelve months we are convinced that this method is a distinct improvement over the two macroscopic tube tests with which it was compared

In a recently published article by Gilbert and Coleman<sup>7</sup> the authors emphasized "the economy of time and glassware," by the use of their presumptive tube test. They further note that the Huddleson rapid method takes "almost as much time as the routine macroscopic tube method." These authors further report that, "the presumptive tests are placed in the water-bath at 55° C for four hours and then left in the refrigerator overnight before being read." Since a Huddleson test requires as a maximum less than five minutes to complete, we question the "economy of time." Further we cannot conceive any economy of glassware since each test requires one pipette only and no test tubes.

In this investigation we again find greater numbers of questionable reactions with the tube tests. Although there were apparently six more positive reactions in the Conn tube test than in the Huddleson test, two of these were tests repeated after a lapse of months and although the tube test still remained positive (1:300), the Huddleson had diminished in titer from 1:3000 to 1:100 where it fell in the "questionable" group under our arbitrary classification. Further, the cases upon which the other four tests were made were not considered undulant fever by the physician. Considering our results from a purely laboratory standpoint we feel that the Huddleson "Rapid Method" will increase the efficiency of our undulant fever diagnostic work allowing us to furnish the physician with an accurate report in a shorter time at no greater expense. On the basis of our results the macroscopic tube test has been discontinued in these Laboratories and the Huddleson "Rapid Method" established as a routine procedure.

By the establishment of an arbitrary standard for reporting specimens as positive, we felt that a dilution of 1:300 showing 50 per cent agglutination was sufficiently high to obviate nonspecific agglutinations. When agglutinins are obtained in such a titer a report of "positive for undulant fever" seems conservative.

In our series of specimens for the year, particularly in the group of specimens 6,686-27,572, we come to an impasse. Either we must consider the 1:300 standard as considerably too low or admit the existence of cases of *Brucella* infection that fail to show recognizable clinical symptoms of undulant fever. It is difficult to conceive with our present knowledge of immunologic phenomena that nonspecific agglutinins in titers as high as the 1:6000 dilution of serum can be present in the blood stream of an individual either ill with "upper respiratory infections" or, more particularly, when no symptoms of any disease exist. Further, it has been reported by Gilbert and Coleman (loc cit) that only 0.4 per cent of 81,848 sera received for complement fixation test for syphilis showed agglutinins for *Brucella* organisms in the 1:80 dilution or higher. These results corroborate those of McAlpine and Mickle<sup>8</sup> who found only 0.6 per cent in a study of 20,000 sera of the same type. Such evidence seems to us to substantiate the value of the positive agglutination reaction as diagnostic of *Brucella* infection.

In the school where the majority of high-titered agglutinins were demonstrated in the absence of clinical symptoms of undulant fever we know that these individuals had been drinking quantities of raw milk from a herd in which abor-



tion had occurred and, further, from which *Brucella* organisms were isolated. In itself this seems sufficient evidence to warrant our findings of *Brucella* agglutinins.

It is true that attempts to cultivate organisms from many of these individuals failed. Even though the demonstration of *Brucella* organisms in the feces or blood stream is an accepted criterion for diagnosis of undulant fever regardless of clinical symptoms, we do not feel that inability to demonstrate the organism precludes the possibility of such a diagnosis. Those who have made attempts to isolate the *Brucella* from man realize the extreme difficulty encountered in most instances. From a study of the literature we find dilutions of 1:80, 1:100, and 1:160 accepted as titers on which to base diagnosis, and so we feel that our 1:300 arbitrary standard is conservative.

The case on which a number of tests were made seems to us to be a definite illustration of a *Brucella* infection in which the symptoms were too obscure and mild to be recognized as such. The case record is reported. Here is an individual who developed agglutinins from a dilution of 1:20 up to 1:6000 and finally decreased to a titer of 1:120. It does not seem probable that a *Brucella* infection did not exist in this case. The patient did have mumps and a slight upper respiratory infection during the year. However, none of the classical symptoms of undulant fever presented themselves at any time during the rise and fall of agglutinins in the blood stream.

The obvious conclusion is that extremely mild cases with atypical symptoms of undulant fever do exist. This may be due to a low virulence of the organism or possibly can be related to the age groupings. Although our records are not sufficiently complete to warrant a definite statement, there is evidence that undulant fever in children and young adults is milder than in the older groups. In conclusion, it seems probable that undulant fever may not necessarily manifest itself with the classical symptoms but may be associated, either directly or indirectly, with upper respiratory infections, coryza, or, as Borman (*loc cit*) notes, middle ear infections.

#### SUMMARY AND CONCLUSIONS

1. The Huddleson "Rapid Method" is an accurate and efficient means for the diagnosis of *Brucella* infection in man.
2. Fewer questionable reactions are obtained with the Huddleson Rapid Method than with the two tube test methods used.
3. A series of cases are presented in which, although no symptoms of undulant fever were demonstrated, high-titered agglutinins were present.
4. *Brucella* infections may be present but not recognizable because of mild symptoms, particularly in young adults and children.
5. This investigation furnishes additional evidence for the substitution of the term "*Brucella* infection" in place of undulant fever.

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## A SIMPLE METHOD OF CHECKING VARIOUS CONCENTRATIONS OF SOLUTIONS OF NOVOCAINE HYDROCHLORIDE\*

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IN VIEW of the not infrequent occurrence of cocaine and procaine poisoning from the use of solutions of higher concentrations than intended, it seems important that a practical test be devised for checking the strengths of solutions of these two anesthetics before use

Such a test in order to be practicable in the pharmacy, operating room or dispensary must be so simple in equipment and technique that it can be carried out by any one without any special training in chemistry, in the minimum of time, and with the minimum of effort Finally, the end point should be sharp and unmistakable

The following test, in our opinion fulfills the above qualifications

*The Test*—The test consists of taking 2 cc of one reagent adding 5 drops of the indicator, shaking then adding 1 cc of the anesthetic solution to be checked, and shaking again The resulting color change is compared with the standard chart The average time for performing this test is thirty-five seconds

*The Reagent*—A N/10 solution of sodium carbonate solution is made according to this formula

Anhydrous sodium carbonate (C P)	53 grams
Distilled water	1000 cc

This solution must be made with fair accuracy Once made, it can be used for a long time

*The Indicator*—In order to cover the wide PH range involved in tests for the

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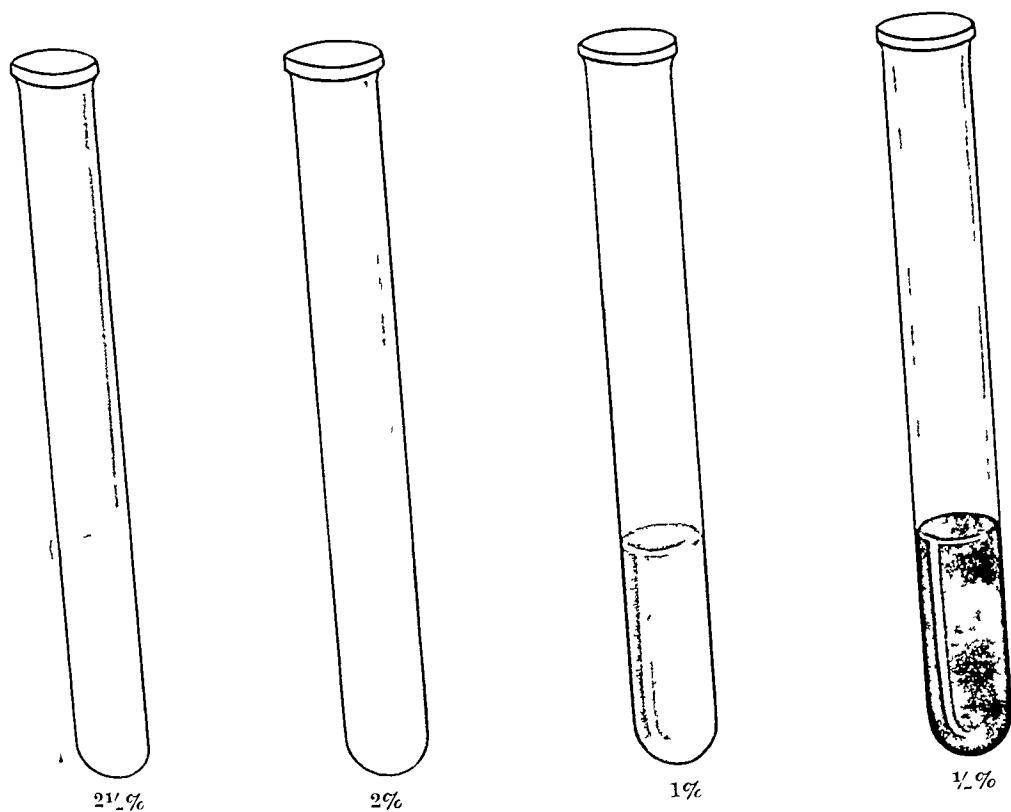


FIG. 1—Color chart for tests with different concentrations of novocaine hydrochloride



common strengths of procaine hydrochloride used in surgical practice, a mixture of two indicators was found necessary. It is made according to this formula

Thymolphthalein	1 0 gm
Phenolphthalein	0 050 gm
Alcohol (70%) to make	100 cc

#### Apparatus —

- (1) Test tubes
- (2) A 2 cc syringe for measuring out the reagent
- (3) A 1 cc tuberculin syringe for measuring out 1 cc of the solution to be tested
- (4) A chart of standard colors which may be made by a local artist

*The Technique* — Was sufficiently described above

*The Color Changes* — The color developed in the tube by the addition of 5 drops of the indicator is deep blue. The color changes resulting from the addition of 1 cc of various concentrations of procaine hydrochloride are shown in Fig 1. Comparison should be made in reflected artificial light.

It will be seen that any concentration higher than 2 per cent renders the resulting mixture colorless. Intermediate strengths cause the development of intermediate shades. A definite color is produced with one drop of the mixed indicator, but because of the fact that the color developed in transmitted light is different from that in reflected light, 5 drops of the indicator are used to intensify the color to such an extent that light transmission through the solution is largely eliminated.

By varying the amount of reagent used or of the solution to be tested, the strengths of solutions above or below these covered by the color range of the chart can be approximately determined. Thus, if a 1 cc solution produces only a slight change, doubt might be aroused as to whether it is a  $\frac{1}{2}$  per cent solution or whether it contains any procaine at all. By reducing the quantity of the reagent to 1 cc, 1 cc of a  $\frac{1}{2}$  per cent solution should bring on a color change similar to a 1 cc of a 1 per cent solution made with 2 cc of the reagent. Again, a solution, 1 cc of which entirely decolorizes the testing mixture, is of a percentage above percentages given in the color chart given, and one is left in doubt as to the actual strength of the solution. If, by reducing the amount of the solution added from the standard 1 cc to  $\frac{1}{2}$  cc or  $\frac{1}{4}$  cc, a color change is brought about that matches that produced by 1 cc of a  $2\frac{1}{2}$  per cent solution in the standard test, then the solution tested is approximately  $\frac{1}{2}$  per cent or 8 per cent. The same result can be accomplished by keeping the solution to be tested at 1 cc, but by doubling or quadrupling the reagent.

Cocaine hydrochloride, because it is somewhat more acid than procaine hydrochloride, produces color changes slightly more marked than, although still comparable with, those produced by procaine hydrochloride.

We wish to thank Dr Isidor Greenwald, of the Department of Biochemistry, and Dr Kenneth C Blanchard, of the Department of Biology, for their valuable suggestions. Grateful acknowledgment is hereby made of the kind encouragement of Professor George David Stewart and of Dr Arthur M Wright, of the Department of Surgery.

## A LABORATORY INHALATION TECHNIC FOR THE COMPARISON OF SEMIVOLATILE LIQUIDS

DAVID A. BRYCE, M.D., PLAINFIELD, N. J.

ATTEMPTS have been made by various independent investigators to develop a standard technic for the absolute and comparative biologic assay of liquids boiling above the body temperature and below 100° C. Fieldner, Katz, and Kinney<sup>1</sup> published an exhaustive study of such liquids, to which I have referred for complete toxicity figures and other data. A similar report was made in conjunction with the Carbide and Carbon Chemical Corporation by the Bureau of Mines, reported through Sayers, Yant, Waite, and Patty,<sup>2</sup> in which they used rather elaborate gas chamber apparatus and gauze wicks, blown by an electric fan, for vaporization. The Chemical Warfare Service has extensive information upon this subject. Photostats of three types of their apparatus have been studied by me, for which I desire to acknowledge the great courtesy of Colonel C. E. Brigham, Commanding Officer of Edgewood Arsenal and Major Linticum of the same post.

In brief it has seemed to me that the various methods previously described have partaken of at least one of the following disadvantages:

- (a) too elaborate and/or costly apparatus
- (b) inaccurate methods of vaporization
- (c) failure to consider adequately the CO<sub>2</sub> content of the gas in the lethal chamber

It has been impossible for me to review the entire world literature upon this subject. The technic herein described is believed to be new, and is certainly original, as it was designed by the writer to solve a laboratory technical problem before any literature on the subject was read. For aid in this work we are indebted to Dr. A. A. Tichnor, Mr. George Hedden, Mr. Lincoln Shafer, and to Mr. Bertus Schnucker for great assistance in conducting the first portion of the work.

The apparatus was designed to provide "open drop" conditions. That is to say, in the lethal chamber there was to be complete and immediate evacuation of carbon dioxide and of the exhaled gases as well as a free sweep for the unexpired gases. This end was obtained by operating the apparatus at a rate of about 1500 c.c. per minute. The factor of adsorption of the various agents to the sides of the lethal chamber or to the skin of the mouse was controlled by frequent cleaning of the chamber with dry tissue, and by using only one mouse at a time in the lethal chamber except in a few of the ether experiments. The apparatus was designed with a view to placing at the disposal of any ordinary chemical or biologic laboratory, by means of routine chemical equipment, a method for the comparative biologic assay of semivolatile liquids.

The apparatus, a diagrammatic sketch of which is attached herewith, may be explained as follows. Flask A is the point of entrance of air at room temperature. In this Erlenmeyer suction flask the bottom is covered to a depth of about two inches with clear, medical, filtered, liquid petrolatum. The intake tube fits just be-

low the surface of this so that air entering the chamber is filtered through the petrolatum and yet does not take up any additional moisture. The air then enters a calcium chloride tube, *B*. From here it passes along the line to a copper coil, *C*, which is immersed in an oil bath. This oil bath is on an electric heater. The air passes from the copper coil along a new line and through a second calcium chloride tube. The air then enters a bead column lying within a steam jacket at a point parallel to the point at which a burette enters the bead column. The burette, *D*, contains the agent with which we worked, and is connected by a tube, *P*, to a compressed air inlet, so as to maintain a positive head of pressure on the liquids at all

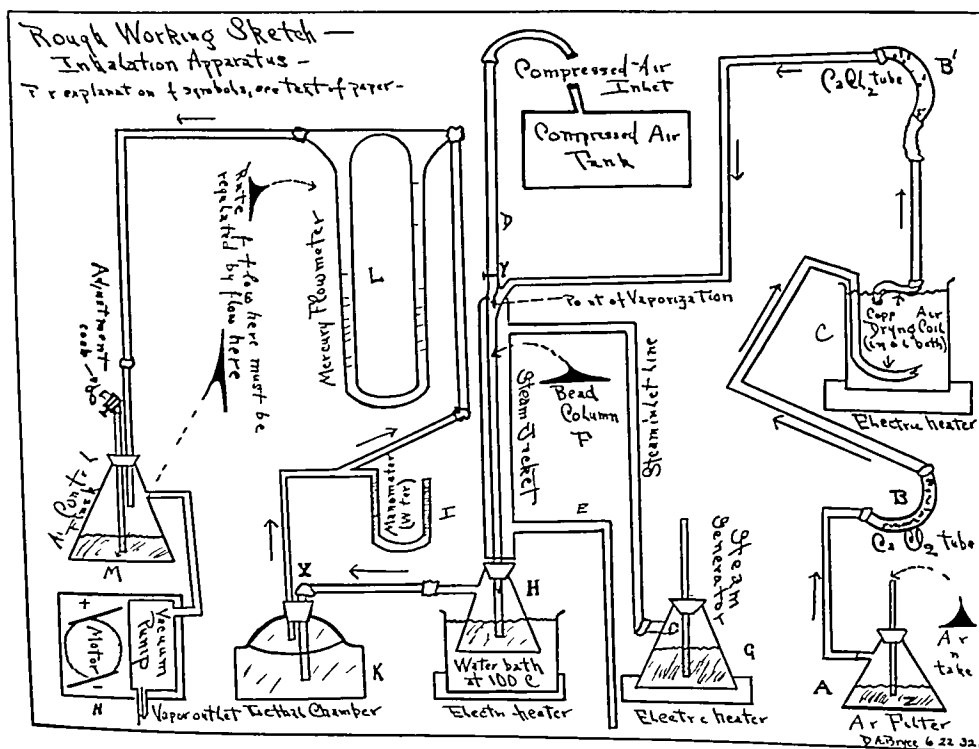


Fig 1

times. The air, and the liquid in minute amounts, pass over the bead column which is heated to any desired temperature up to 100° C by the steam jacket, passing slowly down to vaporize the liquid completely, so that when it enters the Erlenmeyer suction flask, *H*, there should be absolutely no condensation in the tube and none upon the bottom of the flask. The flask is immersed in a water-bath which in turn is heated by an electric heater. Air passes from the Erlenmeyer flask by the suction nozzle and is transmitted directly to the lethal chamber. The latter, *K*, is a small desiccator jar with a perforated rubber cork. The air passes out from the lethal chamber, *X*, to a line into which is placed a water manometer. This manometer, *I*, merely assures proper conditions in the chamber for the mouse. At this point the air proceeds through a flowmeter, *L*, which gauges the evenness and flow of the high vacuum pump. The air goes from this flowmeter to an Erlenmeyer flask which is connected by its suction nozzle *M*, directly to the high vacuum pump,

*N* This flask *M*, serves as a very useful means of regulating the air flow by adjustment of the tube, *U*

It will be noted that by this arrangement standard animals may be weighed and quickly transferred to the experimental chamber under absolutely standard conditions which are constant and controlled, at pressures which are absolutely constant, and under conditions which assure the animal immediate evacuation from the lethal chamber of expired carbon dioxide, and its replacement by an containing its full quota of oxygen. It was found that normal standard mice placed in the lethal chamber with a flow of 1500 c.c. pure air per minute were perfectly comfortable for an indefinite period. Several mice were tried out in this way for periods of forty minutes, and no change was observed.

Certain precautions must be observed in the operation of this apparatus. It will be noted that we have employed an open flame at no point, of course, the reason for this is obvious. At point *Z*, namely the liquid in the air control flask, one must constantly observe the evenness of the flow. This furnishes an index as to whether there is any leakage in the system. If leakage is occurring of course the flow will diminish or cease in the flask. It is not practicable to insert a flowmeter at this point, since if one does so the force necessary to flow through the flowmeter detracts from the final pressure, and changes the reading for the system. At point *X* the stopcock on the burette must be constantly watched. The flow must be adjusted very finely for work of this kind. In our work we used a flow of 0.15 c.c. per minute for most of the work. Point *X* had best be enclosed in a rubber tube of some sort, preferably vulcanized or sealed at the top. Any leakage at *K* or *X* will, of course, invalidate the work, and is promptly apparent in the abnormal action of the air control flask. If these precautions are properly attended to the apparatus forms a convenient and easy method of laboratory procedure.

A note concerning the suitability of white mice for this sort of work may not be ill-timed. In my somewhat brief experience it has appeared that vapors which act entirely or essentially as central nervous toxic agents are well-adapted to use with white mice for toxicity determinations. On the other hand, such liquids as seem to directly affect the heart and kill through this organ, such as chloroform appear to be unsuited for use with white mice in inhalation apparatus. This is a purely preliminary conclusion, if it may even be called such.

The sole excuse of my publishing these notes has been the hope that it may aid other laboratory workers who are faced with some practical problem and find it impossible for economic or other reasons to manufacture an elaborate apparatus involving gas chambers, etc.

#### REFERENCES

- 1 Technical Paper 248, U. S. Dept. of the Interior. Bureau of Mines. Response to Ethylene Dichloride.
- 2 Reprint No. 1349, Public Health Reports 45. 1930.



# NOTE ON THE SUMNER METHOD FOR SUGAR IN URINE\*

JAMES J. SHORT, M.D., NEW YORK, N. Y.

SUMNER<sup>1</sup> in 1925 reported a revised method for the determination of sugar in urine in which he employed the following reagent

To 10 gm of crystallized phenol add 22 c.c. of 10 per cent sodium hydroxide. Dissolve in a little water and dilute to a volume of 100 c.c. Weigh out 6.9 gm of sodium bisulphite and add to this 69 c.c. of alkaline phenol solution. Now add a solution containing 300 c.c. of 4.5 per cent sodium hydroxide, 255 gm of Rochelle salt ( $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ) and 880 c.c. of 1 per cent dinitrosalicylic acid solution. Mix and keep tightly stoppered in well filled bottles.

The method was described as follows:

Pipette into a Folin Wu sugar tube 1 c.c. of urine (diluted if necessary) and 3 c.c. of the reagent. Mix and heat 5 minutes in boiling water. Cool three minutes in running water, dilute to 25 c.c. volume, mix, and compare in colorimeter with standard prepared with 1, 0.5, or 0.25 mg of glucose, according to the concentration of sugar in the urine.

This procedure has been employed by us in the routine analysis of several hundred thousand urines over the past four years. Results have been satisfactory and generally consistent when compared to other standard quantitative methods. For instance the analysis of 100 urines containing sugar in which a comparison was made with Benedict's copper method gave the following averages: Sumner's 2.80 per cent, Benedict's 2.79 per cent. Good agreement was also found in individual analyses. It was found however that with an occasional glycosuric specimen, the reagent gave a result far too low or failed to detect sugar altogether. In one particular case a highly acid urine containing 0.6 per cent sugar gave a result of less than 0.13 per cent. The addition of more alkali to the reagent increased the result to the actual amount. This was reported to Dr. Sumner who, in a private communication suggested the use of 50 per cent more alkali. He also suggested that this might increase the values for normal amounts of sugar in urine. Some experimental results, with increased alkali are reported in Table I.

TABLE I  
RESULTS WITH INCREASED ALKALI ON GLUCOSE STANDARDS

GLUCOSE STANDARD	COLORIMETER READINGS	
	INCREASED ALKALI	ORIGINAL REAGENT
Per cent	mm	mm
0.13	20	22.9
0.20	10	13.0
0.30	10	12.2
0.40	10	10.8
0.50	10	10.9

It will be observed from Table I that increase of alkali definitely increases color production in pure aqueous solutions of glucose.

Analysis of urine specimens containing varying amounts of sugar were made, to compare the results of the original reagent with the one to which additional alkali had been added. The figures are given in Table II. It will be noted that, although in occasional instances there was good agreement, in the majority of instances the results with increased alkali were higher.

TABLE II

URINE SPECIMEN	ORIGINAL REAGENT	REAGENT WITH INCREASED ALKALI	URINE SPECIMEN	ORIGINAL REAGENT	REAGENT WITH INCREASED ALKALI
	per cent	per cent		per cent	per cent
1	0.031	0.046	31	0.140	0.240
2	0.065	0.080	32	0.150	0.190
3	0.067	0.094	33	0.270	0.290
4	0.030	0.048	34	0.240	0.260
5	0.094	0.140	35	0.370	0.370
6	0.065	0.072	36	0.130	0.170
7	0.023	0.027	37	0.250	0.320
8	0.079	0.080	38	0.130	0.170
9	0.098	0.100	39	0.130	0.190
10	0.190	0.210	40	0.140	0.170
11	0.160	0.180	41	0.250	0.280
12	0.180	0.210	42	0.240	0.270
13	0.170	0.170	43	0.120	0.180
14	0.140	0.210	44	0.140	0.190
15	0.150	0.260	45	0.150	0.250
16	0.160	0.230	46	0.150	0.150
17	0.130	0.210	47	0.210	0.260
18	0.120	0.190	48	0.270	0.300
19	0.180	0.230	49	0.210	0.260
20	0.170	0.230	50	0.170	0.210
21	0.250	0.300	51	0.160	0.180
22	0.160	0.180	52	0.140	0.220
23	0.190	0.200	53	0.160	0.250
24	0.300	0.350	54	0.160	0.200
25	0.180	0.250	55	0.120	0.140
26	0.150	0.270	56	0.150	0.180
27	0.280	0.320	57	0.110	0.180
28	0.170	0.190	58	0.120	0.180
29	0.120	0.180	59	0.130	0.150
30	0.140	0.180	60	0.150	0.160

A further comparison was made in which Benedict's quantitative copper method was included. The figures which are shown in Table III, indicate that in undiluted urine the reagent with increased alkali gives results which compare favorably with Benedict's method when the values are between 0.6 and 1.33 per cent, also that the results with the original reagent are generally too low. When urine is diluted even to as small a degree as an equal part with water, the original reagent gives fairly good agreement. Presumably this is because dilution diminishes the concentration of acid. This explains why such good average agreement was obtained between the original Sumner's reagent and Benedict's copper method in our first comparisons on 100 glycosuric specimens as noted above and the need for increased alkali was not then detected. Most of these urines contained sufficient sugar to make dilution a necessity.

TABLE III

UNDILUTED URINE	BENEDICT'S	SUMNER'S STANDARD	SUMNER'S INCREASED ALKALI
1	0.6	0.52	0.58
2	0.6	0.53	0.55
3	1.2	0.71	1.2
4	0.66	0.49	0.63
5	0.88	0.62	0.9
6	1.0	0.79	1.08
7	0.7	0.59	0.65
8	1.0	0.59	0.98
9	1.01	0.66	0.98
10	1.33	0.98	1.23
11	0.7	0.56	0.7
12	1.0	0.57	0.99
DILUTED URINE*			
1	0.7	0.78	0.93
2	0.8	0.82	1.05
3	0.64	0.6	0.71
4	1.1	0.96	1.2
5	1.0	0.9	1.01
6	1.05	0.92	1.15

\*Equal parts with water

Although some results with the increased alkali exceed those with Benedict's reagent and may be somewhat too high, we feel that Sumner's method as modified is in the main reliable and an excellent procedure for routine clinical work. It is our custom to match all tubes with standards in similar tubes and consider those showing reductions of less than 0.13 per cent as having negligible amounts of sugar. Those having amounts between 0.13 and 0.5 per cent are read off and reported directly from the test tube standards. Those above 0.5 per cent are determined by the use of Benedict's copper method.

The writer is indebted to Miss Celia Posen, B.S., and Mr. Arthur Gallagher, B.S., for most of the technical work reported in this article.

## REFERENCE

Sumner, J. B. A More Specific Reagent for the Determination of Sugar in Urine, *J. Biol. Chem.* 65: 373, 1925.

## ADAPTATION OF THE LEITZ ULTROPAK FOR RAPID TISSUE DIAGNOSIS\*

R P CUSTER, M D, PHILADELPHIA, PA

COMPARATIVELY few clinics have perfected frozen section technic to the extent that a fairly accurate pathologic diagnosis is available for the surgeon in five minutes or less, the practical advantage to the surgeon of such a procedure is obvious. The method of Terry seemed to be a step forward in the rapidity with which tissue might be prepared for examination in the clinic not specially trained for frozen section work. Using a special polychrome methylene blue with razor-sectioned fresh tissue, the surface cells were stained and were visible under the microscope by reflected light. In Terry's hands this method proved very satisfactory and fully as accurate a diagnosis could be given in a shorter time than with frozen section, the pathologist not accustomed to interpretation of the type of histologic picture obtained through this medium. However, often felt hesitant to give a definite opinion, illumination difficulties presented an obstacle, the polychrome stain was not clear-cut with tissues containing a considerable amount of fat, for example breast.

We regarded the Ultropak apparatus, recently developed by Leitz as a possible solution to rapid tissue diagnosis, our experiments with it in this direction proved successful.

The Ultropak illuminator (Fig 2), when attached to the microscope stand in place of the usual nose piece (Fig 1), provides illumination of the surface of opaque objects by incident pencils of rays lying entirely outside the path of the rays of the microscope. It is possible to illuminate and examine not only a surface but to view successive strata and deep structures regardless of overlying strata. A wide variety of objectives (dry, water and oil immersion) are available, objectives can be changed rapidly without readjustment of illuminant.

### TECHNIC

1 *Sectioning*—The fresh tissue is cut to a thickness of 1 to 3 mm. with a sharp knife (a light microtome knife or straight razor set aside for this purpose is most satisfactory). The knife is drawn at an angle of 15 degrees from the horizontal rapidly through the tissue, with the distal end of the knife resting on the cork board, care must be exerted to avoid a down-thrust, that would crush the tissue. An effort to obtain a uniformly flat surface is well repaid by the subsequent ease in examination. Extremely soft tissue may be dropped into hot formalin for thirty seconds to one minute to permit greater ease in cutting.

2 *Staining*—The section is placed in a Gooch crucible (or similar container with a perforated bottom) and dipped into a staining dish containing 0.3 per cent

\*From the Division of Pathology of the Philadelphia General Hospital and the Department of Pathology of the University of Pennsylvania.  
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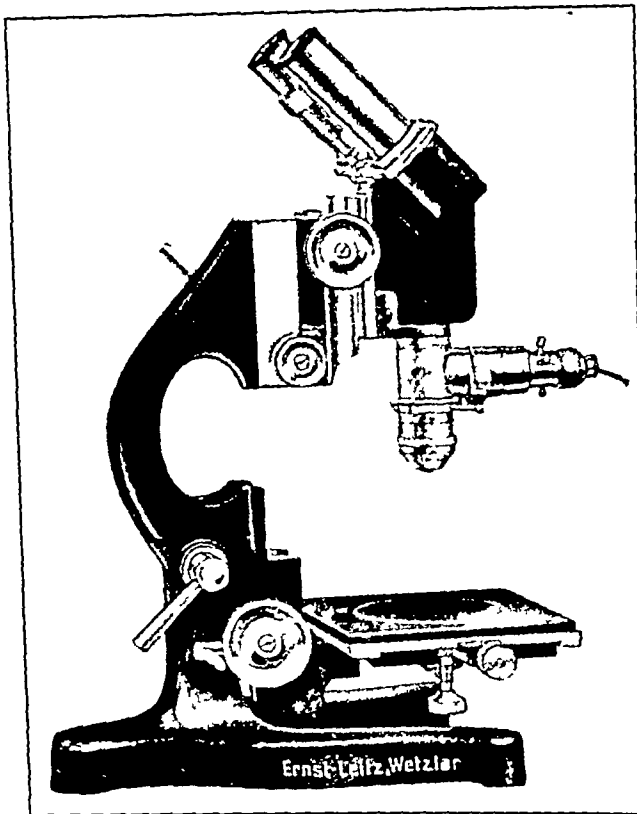


Fig 1

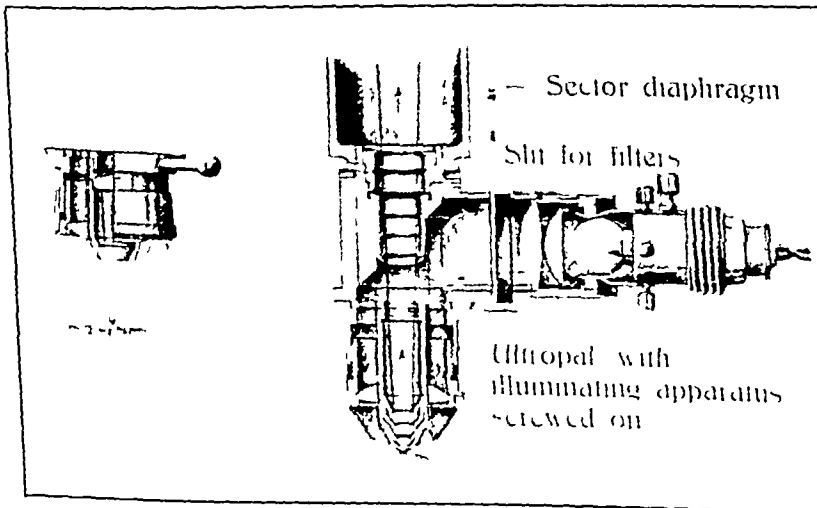


Fig 2

toluidin blue, the section should be moved continuously in the stain and brought into view occasionally that the intensity of the stain can be observed. When the surface is stained a uniform blue (of a degree that must be determined by practice,

requiring usually about twenty to thirty seconds), the crucible is withdrawn and dipped into slightly acidulated distilled water (one drop of concentrated hydrochloric acid to 100 c c water) until the stain ceases to come away in a cloud. The tissue is transferred to a watch-crystal and immersed in distilled water so that the surface is barely covered and the tissue does not float. (This is accomplished by adding water from a pipette until the fluid level approximates the level of the upper surface of the tissue, then placing a final drop directly on the sectioned surface.) The tissue is now ready for examination, the crystal is placed on a white card on the stage. To insure stability of the watch-crystal during examination, it should be cemented to the concavity of a hanging-drop slide.

3 *Examination*—We have found the 65 $\times$  dry objective best for a general outlook over the tissue (the 5 $\times$  with immersion attachment is also useful in some cases). If reflections prove troublesome, a cover slip may be laid across the tissue for low-power examination, being careful to avoid air-bubbles. Sufficiently high magnification for cytologic detail is obtained by removing the cover slip and applying the 23 water-immersion objective. Focus is reached first with the coarse adjustment, sharpness of image being brought out by circular rotation of the objective. The intensity of light must be adjusted with the rheostat control, too low or too great an illumination blurs the field. The Bausch and Lomb (No. 1154) wide-field ocular is an almost essential adjunct.

#### DISCUSSION

The average time required for preparation of tissue for examination by this method is two minutes. The histologic picture is interpreted as readily and accurately as that given by frozen section, a far greater area is available for inspection than with the latter method. Using toluidin blue, nuclei are clear-cut and cytoplasm well outlined, a certain degree of polychrome effect is obtained, in that the stroma takes a pale pink stain as opposed to the blue of the cells. Well-ripened polychrome methylene blue of Unna gave good results but was not as uniform as the toluidin blue, we have had no experience with Terry's polychrome stain, but believe it useful except with fatty tissue.

The following tumor tissues were diagnosed correctly by Ultrapak examination (confirmation of the preliminary diagnoses being obtained by examination of paraffin sections): *Skin* squamous cell carcinoma (Types I, II and III), basal-cell carcinoma. *Lip* squamous cell carcinoma (Type II). *Tonsil* squamous cell carcinoma (Type II). *Lung* metastatic squamous cell carcinoma (Type III). *Stomach* adenocarcinoma (medullary). *Sigmoid* adenocarcinoma (Type II). *Rectum* benign villous papilloma. *Prostate* adenocarcinoma. *Cervix* squamous-cell carcinoma (Type IV). *Breast* adenocarcinoma (Type IV), spindle-cell sarcoma. *Eye* melanoma sine melanin. The single tumor that did not permit positive identification, although its malignant character was obvious, proved subsequently to be lymph node metastasis from an adenocarcinoma of the stomach. Several ulcerative lesions of the skin, diagnosed clinically as malignant, were observed to be inflammatory in character, one of these latter was suspected by Ultrapak examinations to be gumma, this was proved later to be true. Unfortunately, so called "border-line cases" were not encountered during the short period

of time allotted to these observations. Fresh autopsy material (i.e. heart, liver, kidney, etc.) was examined and a rather clear picture obtained, it was found that, in the latter case, fixation in hot formalin gave a better staining reaction and was to be preferred, the time element not being important.

It is hardly necessary to add that increased surety of diagnosis comes through repeated correlation of the histologic picture obtained by the Ultropak and by paraffin section.

I am indebted to Mr. J. P. Vollrath, of Street, Linder and Probert, for the use of the Ultropak apparatus for this work.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**SERUM PROTEINS, In Infancy and Childhood,** Webb, C. H. *Am J Dis Child* 44 1239, 1932

1 Older children have normal values for serum protein at the same level as those in the adult. Infants have low values for total protein and particularly low values for serum globulin, this possibly has some relation to hydrolability or to absence of the infectious diseases.

2 No regular relationship between the state of nutrition and serum albumin content could be established.

3 Acute infections produce comparatively mild and temporary changes in the serum proteins.

4 Alterations in the concentration of serum protein during chronic infections depend on the type of infection. In the series in question the most pronounced changes were seen in (a) chronic osteomyelitis, which was marked by elevated values for globulin, and (b) renal involvement, especially nephrosis, characterized by lowered serum albumin and lowered total proteins but increase in globulins.

**DIPHTHERIA, Immunization Against With Toxoid,** Kientz, P. B. *Am J Dis Child* 44 1249, 1932

Nurses and hospital employees received two 1 c.c. doses of commercial toxoid with seven days between injections. Immunity, measured by a negative Schick test, was present in from 80 to 87 per cent after from fourteen to sixteen weeks.

Immunity, measured by a negative Schick test, was acquired rapidly. One week after the second injection of toxoid, with an interval of one week between injections, from 34 to 43 per cent of the subjects gave negative reactions, at two weeks, from 41 to 46 per cent, at three weeks, from 61 to 63 per cent, at four weeks, from 71 to 73 per cent and at from fourteen to sixteen weeks, from 80 to 87 per cent.

A single injection of 15 c.c. of commercial toxoid did not give sufficient immunity to warrant its continued use in comparison with the immunity obtained by two injections of 1 c.c. of toxoid at an interval of one week.

Data are here presented that tend to show that it is probable that a total dosage of at least 2 c.c. of commercial toxoid is necessary for satisfactory immunization against diphtheria.

**CYANOSIS, In the New Born,** Hunt, F. C. *Am J Dis Child* 44 1268, 1932

By analysis of the results obtained at one hundred and eighteen postmortem examinations, an attempt has been made to ascertain the more common pathologic lesions causing cyanosis in new born infants. It is realized that the causes of cyanosis and of death are not synonymous, and that several factors may be involved. Hemorrhage, whether intracranial, pulmonary or intra abdominal cannot be disregarded as a contributing cause in many instances.

Intracranial lesions, although frequently found at postmortem examination, are not always of sufficient severity to produce symptoms. Especially is this true of small tentorial tears, which must often be disregarded. On the other hand extensive unilateral or bilateral tears of this supporting structure, accompanied as they most frequently are by profuse hemorrhage, would seem sufficient cause for cyanosis. Meningeal bleeding may occur in the absence of tentorial lesions, and is often profusely distributed over the cerebral hemispheres or at the base. These two types of lesion, namely severe tentorial tears and meningeal hemorrhage are



usually the direct result of birth trauma; hence cyanosis will appear within the first few days of life.

The cyanosis accompanying congenital cardiac disease is due to the mixture of venous with arterial blood or to stasis. With such abnormalities, the blueness may vary directly with the activity of the infant and not appear until some weeks after birth. Unless the lesion is severe little is possible in some cases well into childhood. The relatively few cases that appear in this series are therefore not indicative of the true frequency of cyanosis as a manifestation of congenital cardiac abnormalities.

Pneumonia is the most common single lesion found in these infants. The disease does not differ from that seen in older children except for the variability of the signs. The appearance of a hyaline membrane which is probably the result of aspirated amniotic fluid, is more common than was formerly believed. In small amounts, this is probably of little significance. In the cases in which it forms a membrane lining the alveoli of large sections of the lung, it cannot help but cause respiratory embarrassment. Persistent cyanosis appears early in the life of these infants, and may be the only sign present.

Other pathologic conditions may produce cyanosis in the new born, but they are relatively infrequent. The greatest difficulty in diagnosis is usually between pulmonary and cerebral lesions. Lumbar puncture or roentgenologic examination of the chest may be of assistance in establishing the cause of cyanosis but not infrequently may give no definite aid. In the infants coming to postmortem examination, intracranial lesions, including both hemorrhage and tentorial tears, are the most frequent cause of cyanosis, but, as a single severe entity, pneumonia is the predominant factor.

#### BLOOD SEDIMENTATION, Increased Suspension Stability of Erythrocytes, Schulhof, K. J. A. M. A. 100: 318, 1933

A very slow sedimentation rate of the erythrocytes is a constant symptom in allergic individuals unless they show evidence of a complication, especially infection, which tends to accelerate the sedimentation. As an infection may mask the expected slow rate, so may an allergic individual show a slow rate even in the presence of a serious infection of short duration, such as a gangrenous appendix. The interaction of the two opposite tendencies should be taken into consideration in the diagnostic and prognostic evaluation of the sedimentation rates. Sudden postoperative mishap may occur in patients with abnormally slow rates.

Besides the patients with known allergic conditions, a very slow sedimentation rate seems to be fairly constant among patients with lumbago. Liver treatment seemed to account for the slow sedimentation rates in other patients.

The frequency of very slow sedimentation rates among apparently nonallergic close relatives of allergic patients is striking and deserves a closer investigation.

A difference in the technique may partly explain the fact that the slow sedimentation rates have been overlooked clinically.

#### MILK Yeast Medium for Determining the Bacterial Content of, by the Plate Method, Devereux, E. D., and Etcheles, J. L. Am. J. Public Health 23: 149, 1933

This medium differs from plain nutrient agar in that yeast extract and peptonized milk are substituted for meat extract and peptone, and dextrose is added.

##### Formula of Yeast Extract Agar

Yeast extract (Difco)	5 gm
Peptonized Milk (Difco)	10 gm
Salt	5 gm
Dextrose	10 gm
Agar	15 gm
Distilled water	1000 cc

Adjusted to pH 7 and autoclaved at 15 pounds for fifteen minutes.

This medium when used in series with the plain agar described in Standard Methods for Milk Analysis gave counts at the end of twenty-four hours' incubation which were on

the average comparable to plain nutrient agar counts made at the end of forty eight hours, resulting in a saving of twenty four hours for the completion of the test Also it gave counts at the end of forty eight hours which were on the average 45 per cent higher than similarly made plain agar counts

#### MELANURIA, Blackberg, S N, and Wanger, J O J A M A 100 344, 1933

The following method for the detection of melanin in the urine is described as more reliable than those in common use

1 A twenty four hour specimen of urine is evaporated to one fourth of the original volume

2 One gram of potassium persulphate is added for each 100 cc of the concentrated urine

3 At the end of two hours, an equal volume of absolute methyl alcohol is added The precipitated melanin is allowed to settle

4 The precipitate is filtered off and washed with water until the washings are colorless, then washed with methyl alcohol, to remove any soluble pigments remaining Finally, it is washed with ether If the test is positive, there remains on the filter paper a brownish black precipitate, which can be dissolved off with alkali, most conveniently with 5 per cent sodium hydroxide Acidification of the alkaline solution causes a reprecipitation of the melanin

#### PNEUMOCOCCUS, Direct Typing of, Valentine, F C Lancet 224 22, 1933

Referring to the method described by Armstrong (and abstracted in the May, 1932, issue of this JOURNAL), Valentine describes a method whereby the "neutralized" capsules may be made easily recognizable

Three or four loopfuls of the type serum are mixed on a slide with a loopful of sputum and covered with a cover glass the edges of which are sealed with vaseline

After standing for twenty to thirty minutes the cover glass is slid off and the film on the slide allowed to dry The bulk of the vaseline is scraped from the slide and the remainder removed by wiping with xylol

The remaining steps of the procedure are as follows

1 Wash the film gently with tap water (to remove serum)

2 Stain for two to three minutes with dilute carbol fuchsin (1 part of filtered stain to 5 or 6 parts of water)

3 Wash in tap water and counter stain ten seconds with carbol thionin (sat sol of thionin in 50 per cent alcohol 1 part, 5 per cent phenol solution 9 parts Safranin may be used instead of carbol thionin but the staining is less intense)

4 Wash in tap water, blot dry, and examine

Bacteria stain practically black and all else red The capsules of pneumococci treated with homologous serum appear quite large and stain a strong red, "unneutralized" capsules do not stain

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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### Bright's Disease

FOR several years we have in a measure been marking time in our understanding of the pathology and progress of Bright's Disease. This has been due to the fact that by clinical studies we were unable to prognosticate the type of renal lesion that would be found at autopsy. As a consequence the histopathology was to a certain extent neglected by clinicians and attention was directed more to clinical functional studies during life. The work of Addis and the authors of the present monograph has however shown that careful clinical studies during life will enable the physician to visualize the nature of the renal lesion.

The authors accept Addis' clinical classification into hemorrhagic Bright's Disease, arteriosclerotic Bright's Disease and degenerative Bright's Disease. They find that these correspond in general to the pathologic lesions of glomerular inflammation, arteriolar disease and degeneration of the tubular epithelium. They reconcile Addis' clinical observations with the pathological studies of Volhard and Fahr.

The remainder of the volume is devoted to clinical and pathological studies with antemortem functional observations and postmortem structural observations on a series of individuals with Bright's Disease. The authors conclude that the three types of Bright's Disease are essentially different in their genesis and pathological nature. They find, contrary to the general belief, that gradual decrease of urea excreting ability frequently develops during the course of nephrosis. The disease may even end in uremia. In this case the glomeruli are involved in the terminal changes. They conclude, with Addis, that observations during the course of the disease will enable one to deduce the general nature of the pathological changes. Of the different features of the disease that were followed, the blood urea clearance proved to be the most closely related to the onset of final renal failure. The clearance could remain indefinitely at 10 per cent of normal, without uremia, but when it fell below 5 per cent uremia occurred.

This volume together with that by Addis and Oliver, represent the latest and most authoritative expositions on the pathology of Bright's Disease.

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### Cancer†

DR WILLY MEYER, himself a surgeon of eminence, devoted the best efforts of his productive life to a study of the problem of cancer. He recognized that while surgery and x ray and radium are the most helpful means available for combating this affliction, none of them have solved the problem of the control of cancer. This monograph sums up the present situation, reviews the recent advances particularly in the study of the general metabolism and physiology in cancerous individuals, and presents in detail the author's theory of the origin of the disease. He goes further and outlines the program of treatment based upon his theory.

Whether or not the future will show that his theory is correct, his monograph represents a milestone.

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\*Bright's Disease. Observations on the Courses of Different Types and on the Resultant Changes in Renal Anatomy. By D. D. Van Slyke et al. From the Hospital of the Rockefeller Institute for Medical Research. Medicine Monographs Volume XVIII. 66 Charts, four color plates, 36 halftone figures. cloth, pages 130. Williams and Wilkins Company, Baltimore, 1930.

†Cancer. Its Origin, Its Development and Its Self-Perpetuation. The Therapy of Operable and Inoperable Cancer in the Light of a Systemic Conception of Malignancy. A research by Willy Meyer, M.D. Consulting Surgeon to the Lenox Hill and Postgraduate Hospitals, New York Infirmary for Women and Children, etc. Emeritus Professor of Surgery, New York Postgraduate Medical School. Cloth, pages 427. Paul B. Hoeber, Inc., New York, 1931.

The author feels that we will eventually reach a stage at which we can determine the presence of cancerous susceptibility and can inaugurate appropriate treatment prior to the onset of the actual cancer. He feels that cancer is a systemic disease, the resultant of two simultaneously active chronic irritations, one systemic and the other local. Neither alone will produce cancer but both acting in conjunction are responsible for the disease. The systemic chronic irritation has its origin in the irregularities of the nervous system and disturbances of the physico-chemical condition of the body fluids and the local irritation has its origin in an abnormal behavior of the tissue cells. The systemic lesion disturbs the sympathetic-parasympathetic balance thereby upsetting the parathyroid-pancreas balance which in turn alters the normal potassium-calcium ratio of the serum, in favor of potassium. Calcium deficit in turn alters the sympathetic nervous function thereby closing a vicious circle. The result is an increasing serum alkalosis. This produces a hydrophobic condition of the cells, which impairs healing capacity. A person in this condition is cancer susceptible.

The local predisposition also produces a local hydrops and alkalis which tend to prevent healing. The local necrobiotic products stimulate cell reproduction.

He describes four phases in the progress of the disease, the first the phase of cancer susceptibility in which the systemic factors are at work and there is a blood alkalosis, an innocent condition which should be remediable, second the phase of presence of local chronic irritation, also innocent and remediable, the phase of the presence of both systemic and local factors, localized malignancy, operable, and fourth when this phase has been superseded by the additional presence of necrobiotic substances producing cell proliferation and metastases.

The monograph provides ample food for thought and argument.

### Physiology of Bacteria

**A**SIDE from Buchanan and Fulmer's *Physiology and Biochemistry of Bacteria* which has been reviewed in these columns and Stephenson's *Bacterial Metabolism*, there is no other up to date work on the subject. The volume under review is the third contribution in this field. The first of the three was written primarily as a reference book and the second emphasized only one phase of bacterial physiology, metabolism. The present writer presents a coordination of the various simplest functions of life with a study of each function and its effect upon the other functions. Attention is devoted primarily to the necessary functions of life. To this extent it presents a study of the necessary functions of life as applied to the simplest forms.

Subject matter treated includes endogenous catabolism, energy supply, growth, and the mechanism of death. The volume is a critical summary and review of the literature and appears to be very complete. Undoubtedly it will find a large field of usefulness among bacteriologists and among plant and animal physiologists.

### Pathology, Bacteriology and Applied Immunology for Nurses†

**A**FEW years ago this author published a small handbook on bacteriology and immunology for nurses which was reviewed in this column. The present volume adds a section on pathology. Pathology is divided into two sections, general and special. The first section discusses the causes of disease, the classification of disease and the pathologic changes resultant on the action of the causes. The second section describes the system diseases in sufficient detail so that nurses, technicians and others taking similar types of courses can acquire an adequate general comprehension of what is going on in the patient. The section on pathology as well as that on bacteriology, previously reviewed, is abundantly and excellently illustrated.

\*Physiology of Bacteria. By Otto Rahn Professor of Bacteriology Cornell University Ithaca, N. Y. Pages 438 with 42 illustrations cloth. P. Blakiston's Son and Company 1912 Walnut Street Philadelphia 1932.

†Pathology, Bacteriology and Applied Immunology for Nurses. By Robert A. Kilduffe A. B. A. M. M. D. F. A. S. C. P. Director Laboratories Atlantic City Hospital. City Bacteriologist Atlantic City. Serologist Municipal Hospital for Contagious Diseases Atlantic City. Pathologist Atlantic County Tuberculosis Hospital. Serologist Jewish Seaside Home Atlantic City etc. Formerly Major M. C. U. S. A. Cloth pages 324. The Bruce Publishing Company, New York 1931.

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Editor WARREN T VAUGHAN, M D  
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## EDITORIAL

### The Renaissance of Morgan's Bacillus No I

THE exact degree of pathogenic importance to be ascribed to the organism first isolated from the feces in cases of summer diarrhea in children by Morgan<sup>1</sup> in 1906 has long been a matter of uncertainty. Relatively recent developments, however, have again brought this organism to the fore and render a review of its present status of interest.

As not only its pathogenic importance but also its bacteriologic identity have been the subject of contention, the present discussion may well be prefaced by a brief description of the organism itself.

The organism first isolated by Morgan in twenty-eight of fifty-eight cases may be described as a slender, gram-negative rod averaging 1 to 3 micra in length when studied in young broth cultures but often exhibiting large and bizarre involution forms in older agar cultures.

Usually slightly motile it may occasionally be immobile. The organism produces indol, most vigorously in young cultures. Gelatin is not liquefied nor milk coagulated. When grown on acetate-agar a brownish discoloration is produced. No growth occurs on citrate agar. Fermentation studies show consistent acid formation with the production of gas in levulose, galactose-peptone water, and glucose, although in the last gas production may be so slight as to be detected only with difficulty in deep stab glucose agar cultures, and occasionally may even be absent.

As originally described by Morgan<sup>1</sup> and later studied bacteriologically by Morgan<sup>2, 3</sup> and Morgan and Ledingham<sup>4</sup> the organism was grouped in accordance with its fermentation reactions as shown in the table following.<sup>5</sup>

GROUPING OF MORGAN'S BACILLUS IN ACCORDANCE WITH FERMENTATION REACTIONS

	GLUCOSE	MANNITOL	DULCITOL	LACTOSE	SUCROSE
Type I	AG*	O**	O	O	O
Type Flexner	A	A	O	O	O
Type V	A	O	O	O	O
Type Goertner	AG	AG	AG	O	O
Type X	AG	AG	AG	O	AG
Type XII	AG	AG	O	O	AG
Type XIV	AG	AG	O	O	O
Type XV	O	O	O	O	O

\*A = Acid G = gas

\*\*O = no reaction

It is quite apparent that the organism is a member of a group of closely related bacteria capable, however, of differentiation by means of their fermentative and also, as shown by other and later workers, their serologic characteristics.

Perhaps the most extensive and thorough bacteriologic study of this group, since its original description, is that made by Thjøtta<sup>6</sup> who concludes from a thorough morphologic and cultural study, as well as from extensive serologic investigations (agglutination and complement fixation tests), that the Morgan bacillus is simply a *Bacterium coli* of a certain fermenting type and who proposes, therefore, that it be more properly known as *Bacillus metacoli* (Morgan).

In this view Thjøtta is supported by d'Aunoy.<sup>7</sup>

The bulk of the discussion concerning this organism has not centered upon its bacteriologic identity but upon its ability to produce disease.

Although definitely associated by Morgan, as well as by others, with the production of that form of infectious diarrhea formerly frequently spoken of as "summer complaint" or "summer diarrhea of infants," this assumption has been attacked by many observers.

The principal basis of this disagreement has been two-fold and arises, first, from discordant results of studies of the pathogenicity of the organism for laboratory animals, and, second, upon the failure to demonstrate agglutinin production in the blood of the patients from whom the organism was isolated in connection with processes of which it was probably the cause.

Perhaps because of this uncertainty the organism has not been specifically and consistently looked for but, nevertheless, evidence has been gradually accumulating which purports to confer upon the Morgan bacillus a definite pathogenic

importance and which, moreover, suggests that the organism is not only widely distributed, but also that the pathogenicity for man, of some strains, at least, may be increasing

The literature on this subject has been extensively reviewed by MacKenzie and Batt<sup>8</sup> from whose paper much of the data to follow has been abstracted

Thus evidence of the pathogenicity of Morgan's bacillus for mice has been reported by Wilson<sup>9</sup> and for mammals, birds, and reptiles by Lovell<sup>10</sup>

Efforts to demonstrate the presence of homologous agglutinins where Morgan's bacillus has been isolated from man have met with but inconstant success

Their occurrence in five of thirteen cases in which the clinical picture was that of paratyphoid fever but blood cultures were positive for Morgan's bacillus has been reported by Havens and Ridgway,<sup>11</sup> and Waaler<sup>5</sup>

While this angle of the controversy still remains to be elucidated and deserves, as it will doubtless receive, further study, clinical and bacteriologic evidence of the pathogenicity of Morgan's bacillus for man has been the subject of many relatively recent reports

Dick, Dick, and Williams<sup>12</sup> for example, report an epidemic of enteritis associated with mastoiditis in infants which they regard as due to this organism, and a similar association of pseudomembranous enterocolitis and mastoiditis with the isolation of Morgan's bacillus from the middle ear and heart blood at autopsy is reported by Sutton<sup>13</sup>

Thirteen cases presenting clinical symptoms in common, characterized by sudden onset, usually with a chill and an equally abrupt termination, are reported by Havens and Ridgway<sup>11</sup>

These were encountered in Alabama and occurred in adults as well as children

Positive blood cultures and the presence of homologous agglutinins were found in five cases, Morgan's bacillus being isolated from either the blood or feces in all the cases of the series

d'Aunoy<sup>7</sup> reports three cases of pyrexia, pyelitis, and colitis in which Morgan's bacillus was isolated, one strain being pathogenic for guinea pigs and white rats, homologous agglutinins being demonstrated in the blood of all three patients. These cases occurred in Louisiana

An epidemic outbreak in a small rural community in New York State involving ten children from one to fifteen years of age is reported by MacKenzie and Batt<sup>8</sup> in which the primary source of the infection was a contaminated water supply, flies, or direct transmission being responsible for the further spread of the disease

Five cases of urinary tract infection, four in adults and one in an infant one year old are reported by Waaler,<sup>5</sup> Morgan's bacillus being isolated from all and regarded as the etiologic agent concerned

Intestinal infections in which Morgan's bacillus is regarded as having some part have been reported by Silverman and Harris<sup>14</sup> and by Mac Gill and Downie,<sup>15</sup> and Pickles,<sup>16</sup> and, finally, Gittins and Hawksby,<sup>17</sup> report the isolation of Morgan's bacillus from the blood and spinal fluid in two cases, one fatal, of meningitis associated with bacteremia

The evidence thus briefly summarized suggests not only that, as already recognized, Morgan's bacillus has an extremely wide distribution but also indicates a

very definite pathogenicity for man and suggests in addition that this ability may be on the increase

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## CLINICAL AND EXPERIMENTAL

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### THE HEMORRHAGIC TENDENCY IN JAUNDICE A STUDY OF THE BLOOD FIBRIN, SEDIMENTATION RATE, COAGULATION TIME, AND OTHER BLOOD FACTORS\*

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CHARLES F BURKE, M D, MADISON, WIS, AND JAMES F WEIR, M D,  
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THE hemorrhagic tendency of the jaundiced patient sometimes causes much concern. By the careful preoperative preparation outlined by Walters,<sup>37 38</sup> the operative mortality in jaundice has been reduced. However, there are patients who tend to bleed in spite of these preventive measures. Little is known regarding this hemorrhagic tendency. In an effort to add to knowledge of this subject, various aspects of the blood of jaundiced patients were studied as well as of other patients who could be considered as controls. These studies included blood fibrin, sedimentation rate of erythrocytes, coagulation time of the blood, and bilirubin, protein, and viscosity of the serum. Only cases of jaundice of obstructive or intra-hepatic type have been considered in this work.

#### METHODS AND NORMAL VALUES

In our study, the method of Foster and Whipple was used for determinations of blood fibrin, except that "oxalated tubes" were used instead of sodium oxalate solution. These tubes were prepared in the following manner: Of a 3.2 per cent solution of sodium oxalate, 1 c c was added to each of the 15 c c graduated centrifuge tubes. These were then placed in a sand bath, and in the process of drying, the sodium oxalate solution was spattered on the sides of the tubes. Another vari-

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\*Abridgment of thesis submitted by Dr. Burke to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine. Work done while a Fellow in Medicine. The Mayo Foundation.  
Received for publication May 26 1932

ation from the method of Foster and Whipple concerned precipitation and collection of the fibrin. When the fibrin was completely coagulated it was freed from the walls of the tube by a glass rod. The tube was then centrifugalized at a rapid rate, until the fibrin was closely packed in the bottom of the tube, in a button-like formation. In the remaining details then method was closely followed.\* We found that in men the concentration of fibrin ranges normally between 170 and 340 mg., and in women, between 280 and 360 mg. in each 100 cc. of blood. These values are somewhat higher than those noted by Foster.

In our work on sedimentation of erythrocytes we followed the method of Plass and Rourke with the exception of one aspect of it, because of its simplicity and because heparin is used for the anticoagulant. Instead of using collection tubes prepared as described, we added to each collection tube, immediately before it was used, 0.1 cc. of a 3 per cent solution of heparin (containing 3 mg. of anticoagulant). This was done because the heparin, dried in the tube as suggested, did not uniformly prevent coagulation. The normal range of sedimentation rate for women, determined by us, was 3 to 29 mm. per hour, or 5 to 58 per cent. For men it was 2 to 18 mm. per hour or 5 to 34 per cent. These figures are somewhat higher than those of Plass and Rourke.

The method of Lee and White was used in our study for determination of the coagulation time of the blood. Lee and White gave as the average normal coagulation time six and a half minutes, with five to eight minutes as the limits of normal. Our results were similar for normal persons. We have arbitrarily refused to consider the coagulation time sufficiently prolonged to be of clinical significance if it is less than twelve minutes.

Van den Bergh concluded that two types of reaction were seen in determinations of serum bilirubin by his method: one was obtained immediately after addition of the diazo reagent, a "direct reaction," and the other after addition of the diazo reagent and alcohol, an "indirect reaction." Two other types of reaction are described which are probably not of clinical significance, the "delayed reaction" and the "biphasic reaction." The delayed reaction commences only after one to fifteen minutes or longer, and consists in the development of a reddish coloration, which gradually deepens and becomes more violet. In the biphasic reaction a slight reddish color appears immediately (ten to thirty seconds) which, after a minute or a much longer time, deepens gradually and becomes more violet. The delayed reaction is reported as "indirect" and the biphasic as "direct." The Thannhauser and Anderson modification of the indirect van den Bergh reaction is used in the laboratories of The Mayo Clinic for quantitative determination of the amount of bilirubin in the blood serum. The results are reported in milligrams of bilirubin for each 100 cc. of serum.

For determination of proteins of the serum, the refractometric method of Reiss, as modified by Neuhausen and Rioch, was used. The viscosimeter of Hess was employed for determining the viscosity of the serum. Bucher gave 1.7 to 2.0 as the normal range for viscosity of the serum. Our values for normal persons fell within these limits.

\*A few of our determinations of fibrin were made by the method of Schultz, Nicholes, and Schaefer. The difference between the results of the two methods is of little consequence.

## CORRELATION OF FACTORS IN JAUNDICE IN REGARD TO HEMORRHAGIC TENDENCY

Snell, Greene and Rowntree studied the blood fibrin of experimental animals in which obstructive jaundice was produced. They found a sharp rise postoperative in all animals, with gradual return to normal for several days. Thereafter the values approached the upper limits of normal as a rule. Occasionally high values were observed, but in no instance was there a definite decrease in fibrin. They observed no direct relationship between coagulation time and fibrin content of the blood. In each of four cases of obstructive jaundice due to carcinoma, Gram found high values for blood fibrin. Of four cases of catarrhal jaundice, in only one was there an increase in fibrin. In another series, however, Gram found the blood fibrin increased in four cases of catarrhal jaundice. Mann, Bollman, and Markowitz attributed the delayed coagulation time in dogs following hepatectomy to deficiency in fibrinogen of the blood. However, they found no simple relationship between the content of fibrinogen of the blood and the coagulation time. According to Stajlinger, the fibrinogen and globulin of the blood in jaundice seem to be increased, except in grave icterus in which the fibrinogen is greatly diminished.

The sedimentation rate is influenced by many factors. Fåhræus noticed pronounced parallelism between the amount of fibrinogen in the blood, or the serum globulin, and the sinking velocity of the corpuscles. Hunt<sup>21, 22</sup> also found a definite relationship between the fibrin content of the blood and the rate of sedimentation.

A similar relationship has been noted by many other workers, including Greisheimer,<sup>6, 7, 8</sup> Ryan and Johnson, Gram, and Rouike and Plass. On the other hand, Pinnet, Knowlton and Kelly have expressed the belief that there is no definite relationship between the blood fibrin and the sedimentation rate of the erythrocytes. The sedimentation is affected, also, by the number of erythrocytes, the cell volume, and the concentration of hemoglobin. Hunt<sup>21, 22</sup> could establish no definite ratio between the erythrocyte count and the sedimentation rate, nevertheless, he expressed the belief that variation of the cell count to any great extent, either above or below normal limits, affected the sedimentation test. By decreasing the cell volume he found that normal sedimentation became more rapid, and by reversing this process, that normal sedimentation became slower. His opinion was that the sedimentation rate was influenced by anemia to such an extent that it could not be relied on in such cases. Rubin and Smith, using hirudinized blood, noted close relationship between the erythrocyte count, the sedimentation rate, and the cell volume as determined by the hematocrit. The lower the cell volume the greater was the rapidity of sedimentation. Similar relationships have been found by other workers, including Hubbard and Geiger, Gram, and Greisheimer, Ryan and Johnson. Various methods have been described for the correction of the sedimentation rate made inaccurate by variations in number of erythrocytes, concentration of hemoglobin, and cell volume. The methods of Plass and Rouike, and of Gram, deserve mention. Greisheimer discovered no significant difference in the sedimentation rate during the menstrual flow from that in the intervals between periods of flow. Lee and White, in 1913, suggested a relationship between the coagulation time and the settling of the corpuscles as demonstrated by the "buffy coat." Ro-

senthal and Blowstein found the sedimentation rate increased in 80 per cent of the cases of jaundice in their series. They expressed the belief that the sedimentation rate has no relation to the degree or duration of jaundice or of biliary obstruction. They noticed normal sedimentation rates in a number of cases of catarrhal jaundice, and concluded that a normal sedimentation time, if a patient were jaundiced and without polycythemia, is suggestive of catarrhal jaundice. Linton noted correlation between the sedimentation rate and the hemorrhagic tendency in jaundice. In his work he divided postoperative hemorrhage among patients with obstructive jaundice into primary or early hemorrhage occurring immediately after operation, and secondary or delayed hemorrhage becoming manifest a week or ten days after operation. He found that the preoperative sedimentation rate in three of four cases in which there was primary bleeding was extremely rapid. In ten cases in which there was no primary hemorrhage the sedimentation rate was slow in all except one, and in this case fever was present. The sedimentation rate was taken at the time of the hemorrhage in seven cases. The rate in all cases was very rapid. Only one of the patients had fever, which might have accounted for the increased sedimentation rate. Postoperative determinations gave some indication as to the possibility of delayed bleeding. Linton expressed the belief that the sedimentation rate of the blood is a more reliable test of the hemorrhagic tendency in jaundice than determinations of coagulation time and of bleeding time.

The coagulation time has always been studied in consideration of the hemorrhagic tendency of jaundice. The belief expressed by Wangensteen is that the bleeding tendency does not hinge on decreased coagulability. According to Linton, determination of the coagulation time preoperatively is of little value in the prognosis of postoperative bleeding in obstructive jaundice. Snell, Greene and Rowntree found that following the production of obstructive jaundice in dogs there was in general fairly close parallelism between the degree of retention of bile and the coagulation time, although in some animals spontaneous reduction in the coagulation time was noted late in the progress of the condition.

The degree of jaundice, as demonstrated by the values for serum bilirubin, is of importance in regard to the hemorrhagic tendency according to Walters<sup>39</sup> and Judd. Although it is well known that hemorrhage may be manifest in jaundiced patients with low values for serum bilirubin, it is also conceded that bleeding is anticipated more when the value for serum bilirubin is high.

Values for protein and viscosity of the serum are also considered in this work. Snell and Greene noted that following prolonged obstruction of the biliary tract, experimentally produced in dogs, there was progressive reduction in the proteins from 7.1 to 5.4 gm. for each 100 cc. of serum. In regard to the viscosity of the serum, Groedel and Hubert found no parallelism between the speed of sedimentation of the corpuscles and viscosity of the serum.

Calcium of the blood has been extensively studied in jaundice. Despite earlier work to the contrary, Vines asserted, in 1921, that the presence of ionized calcium is not essential to the process of clotting. In studies of calcium made on jaundiced patients and experimental animals, Snell and Greene found no significant disturbance either in the amount or the proportion of diffusible calcium in the serum in the presence of jaundice. There was no correlation between either the total or

the diffusible calcium content of the serum, and either the serum bilirubin or the delay in the coagulation time of the blood in jaundice. They concluded that "jaundice does not produce a clinically significant disturbance in either the quantity or condition of the calcium in the serum of adult dogs or hospital patients." Because of these thorough studies, blood calcium was not considered in this work.

The duration of the jaundice is thought by many to have significance in regard to the tendency to bleeding. Wangenstein has noted that in most instances in which the bleeding has come under observation together with abnormalities of coagulation, the biliary obstruction has existed for some time. Of 58 of Petren's patients who died because of postoperative hemorrhage, 50 had jaundice for three weeks or more. Judd and Walters<sup>39, 40</sup> also expressed the belief that there is a relationship between the duration of the jaundice and the hemorrhagic tendency. Mayo-Robson noted increased liability to bleeding when the obstruction is due to carcinoma.

#### HYPOTHESES REGARDING THE ETIOLOGY OF THE HEMORRHAGIC TENDENCY

Many hypotheses have been expounded in regard to the etiology of the hemorrhagic tendency in jaundice. Retention of bile is thought by some to be a factor. Wangenstein expressed the belief that retention of bile probably is responsible for the bleeding. Snell, Vanzant, and Judd have found no correlation between the coagulation time and the values for bile acids or bilirubin. They considered, also, lipoid substances retained in the blood in jaundice but did not believe them to be etiologic. Destruction of hepatic tissue and diminution of hepatic function consequent on biliary obstruction might be considered the chief etiologic factors, according to Wangenstein. Disturbance of calcium metabolism is suggested by the work of Walters and others. Pawlow found that in dogs with complete biliary fistulas osteoporosis frequently developed. Seidel found the same to occur in some cases of biliary fistula. McCrudden noted that osteoporosis developed with continued biliary obstruction. These facts indicate that there may be some disturbance of metabolism of calcium in jaundice. On the contrary, are the findings of Vines, of Ravdin, Riegel and Morrison, and of Snell and Greene, which are equally convincing. The studies of Ravdin, Riegel and Morrison suggest a disturbance of metabolism of carbohydrate. On the administration of glucose intravenously or by stomach tube to jaundiced experimental animals they noted marked reduction in the coagulation time of the blood of each animal. Of four of six jaundiced patients the coagulation time was reduced by administration of glucose. They suggested that glucose may produce its effect by its reparative action on the liver. Partos and Svec reported exact parallelism between the coagulation time and the content of blood sugar. In the experience of Ravdin, Riegel and Morrison the coagulation time and the values for blood sugar do not consistently parallel each other, although reduction in the coagulation time is frequently associated with an increase in blood sugar. Delayed coagulation of the blood often is blamed for the hemorrhagic tendency. However, it is known that hemorrhage may occur in jaundice in the presence of normal coagulation time. Snell, Vanzant, and Judd suggested that hemorrhage in jaundice may be due to excess of heparin (antiprothrombin) in the blood, which may be produced by injury to the liver, or which may be due to decreased destruc-

tion by the liver of heparin formed elsewhere. However, since there is no method for determining heparin quantitatively in the blood, this cannot be proved.

#### OBSERVATIONS

Our study concerned 46 patients with jaundice, also, it included 21 patients without jaundice or hepatic disease who could properly be considered as controls. All determinations were made preoperatively. One or two of each of the various tests were done on each patient.

In this series, values for blood fibrin were found to be increased in jaundice in the majority of cases (Tables I, II, and III). Thirty-four of 43 jaundiced patients (79 per cent) had values for blood fibrin above the upper limits of our normal ranges. We could not demonstrate any definite relationship between the blood fibrin and the coagulation time. Mann, Bollman, and Markowitz had similar results. No correlation was seen between the blood fibrin, and the value for serum bilirubin. The content of fibrin of the blood revealed no relationship to the leucocyte count or to the values determined by hematocrit.

A fairly constant relationship was seen between the values for blood fibrin and the sedimentation rate of erythrocytes. This had been previously noted by many workers. The sedimentation rate was found to be increased in jaundice, and no difference was noted among the various types of jaundice. Of our 46 jaundiced patients, 43 (93.4 per cent) had sedimentation rates above normal (Tables I and II). Normal values are given in Table III. The sedimentation rate was influenced by an abnormal decrease in the number of erythrocytes, in the percentage of hemoglobin, or in the hematocrit values. This has been noted frequently by others. There was no simple relationship between sedimentation rate and the values for serum bilirubin. No relationship could be demonstrated between the sedimentation rate and the coagulation time, or the sedimentation rate and the leucocyte count.

A study of the values for protein and viscosity of the serum revealed nothing of significance, and there was no relationship between them and other factors of the blood. However, Snell and Greene found that in jaundice of long duration in experimental animals, elevation of the values for serum bilirubin became less pronounced, and the values for serum protein were decreased. The values for serum protein were decreased, also, in cases of severe malnutrition. Although these patients had lost varying amounts of weight, the reduction was not sufficient to cause changes in the values for serum protein.

Of the 46 jaundiced patients 20 (43.5 per cent) gave evidence of a hemorrhagic tendency demonstrated by purpura, petechiae, epistaxis, blood in the stools, bleeding from the gums, vomiting of blood, or the finding of blood in the peritoneal cavity. From the standpoint of sex it is interesting to note that the 7 patients with purpura were females.

Six of the 10 patients (60 per cent) whose obstruction was due to carcinoma had a tendency to bleed. This is a higher percentage than was seen in the cases of obstructive jaundice due to other causes and would indicate that in cases of carcinoma the tendency to hemorrhage is greater. Mayo-Robson expressed this same belief. However, the number of our cases was much too small to warrant positive conclusions.

CASES WITH JAUNDICE AND WITH HEMORRHAGIC TENDENCY

CASE	SEX	DIAGNOSIS	DURATION OF JAUNDICE	MANIFESTATIONS OF HEMORRHAGE	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG IN EACH 100 C C OF SERUM*	COAGULATION TIME		FIBRIN, MG IN 100 C C		SIDEROPHILIC INDEX		HEMATOCRIT		PROTEIN OF SERUM, GM	VISCOSITY OF SERUM
								MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MILLIMETERS	PER CENT	ON PLATE, PER CENT	HYPATIN, PER CENT		
1	M	Carcinoma of pancreas	3 weeks	Postoperative bleeding	4.46	55	26.7	11	30	522	855	15.0	25.9	37.8	42.2	75	2.1
2	M	Carcinoma of gallbladder	2 weeks	Seen at necropsy	4.48	69	12.0	9		559	825	12.5	60.0	39.0	36.0	86	2.0
3	M	Carcinoma of pancreas	4 weeks	Blood in stools	3.07	18	11.0	5	15	550	792	50.5	78.9	30.5	36.0	58	1.9
	M						11.6	6								68	2.0
	M						11.6	6								65	1.4
4	F	Carcinoma	5 weeks	Purpura, bleeding from gums	2.00	36	16.8	11		477	627	70.3	91.2	23.9	23.0	61	1.9
	F						16.2	14		469	612	65.0	90.2	27.0	27.5	82	2.2
5	F	Carcinoma	5 weeks	Epistaxis, purpura, po- technic, blood in stools	1.50	73	28.1	12		471	748	39.0	70.0	37.0	35.5	82	2.2
	F						23.2	7		501	645	19.0	71.0	22.4	30.5	75	2.2
6	F	Carcinoma of gallbladder	9 weeks	Purpura	3.80	60	20.0	8		295	415	47.0	81.0	37.5	42.0	75	2.2
	F						13.4	9		435	670	44.0	76.0	35.0	42.0	82	2.2
7	M	Stricture of common bile duct	6 months	Blood in stools, post operative bleeding	3.86	63	13.4	9									
	M						5.2	9		328	540	61.0	88.0	30.1	38.7		
8	M	Stricture of common bile duct	1 month	Epistaxis	1.10	65	5.2	8	35	440	687	36.5	64.6	30.0	43.5	78	2.2
9	F	Stricture of common bile duct	8 months	Purpura	1.40	72	21.2	10									
10	F	Stricture of common bile duct	5 months	Postoperative bleeding													
	F						11.3	16		571	725	58.0	91.0	24.0	36.0	75	2.4
11	F	Stricture of common bile duct	2 years	Vomiting of blood	2.82	50	11.3	16		301	437	34.5	55.0	31.0	40.5	73	2.0
	F						10.3	10									
12	F	Stone in common bile duct	3 months	Postoperative bleeding	3.64	65	10.3	10	30	585	852	41.0	73.0	31.0	40.0	67	2.0
	F						13.0	11									
13	F	Stricture of common bile duct, biliary cirrhosis	14 months	Purpura	3.71	68	13.0	11		771	870	32.0	55.0	34.0	42.0	70	2.0
14	F	Stricture of common bile duct	4 weeks	Purpura	3.28	55	21.1	15									
	F						17.6	6		619	950			31.8		81	2.2
15	F	Stone in common bile duct	1 weeks	Purpura	3.06	52	17.6	6	30	403	670	35.3	61.0	39.8	42.2		
	F						22.4	6		461	690	59.2	86.5	32.7	31.6		
16	F	Chronic cholecystitis with choledithiasis	7 weeks	Purpura	1.92	76	1.3	8									
	F						27.0	7		386	690	12.0	75.0	14.0	44.0	73	2.0
17	F	Stricture of common bile duct, biliary cirrhosis	2 months	Purpura, epistaxis	4.94	78	23.0	7	10	413	750	35.0	63.6	44.5	45.0	51	2.2
	F						39.0	11		369	561	27.5	46.5	31.3	11.0	69	2.0
18	F	Subacute yellow atrophy of liver	1 week	Pretchae	1.60	72	39.0	11	40								
	F						3.9	8		254	410	18.8	81.3	42.1	40.0	51	2.4
19	F	Infective hepatitis	1 year	Purpura	4.03	68	4.8	8	30	216	370	48.9	82.8	11.4	41.0		
	F						31.2	6		319	590	33.2	59.6	10.8	11.3		
20	F	Portal cirrhosis	1 month	Purpura	4.16	68	31.2	6									

\*Van den Bergh reaction direct

TABLE II  
CASES WITH JAUNDICE BUT WITHOUT HYPOMURHAIC TENDENCY

CASE	SEX	DIAGNOSIS	DURATION OF JAUNDICE	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG IN EACH 100 CC OF SERUM*	COAGULATION TIME		BILIRUBIN, MG IN EACH 100 CC	SEDIMENTATION RATE		HEMATOCRIT		PROTEIN OF SERUM, GM	VISCOSITY OF SERUM
							MINUTES	SECONDS		PLASMA	MILLIMETERS	PER CENT	OXALATE, PER CENT		
21	M	Carcinoma of biliary ducts	3 months	4.19	70	18.8	9	30	417	613	47.2	76.5	32.0	7.0	2.0
22	M	Carcinoma of pancreas	1 month	1.72	16	10.0	14		500	588	85.0	94.0	15.0	6.6	2.0
23	M	Carcinoma of pancreas	6 weeks	4.61	75	43.9	6	30	452	637	30.0	46.0	29.0	6.5	1.9
24	M	Carcinoma of pancreas	2 weeks	4.16	66	15.5	8				28.0	46.0	37.0	6.6	1.9
25	M	Acute cholecystitis with stones	7 days	4.60	70	2.1	9		623	1060	42.0	80.7	41.2		
						2.3			767	1252	50.0	85.9	38.7		
26	M	Chronic cholecystitis	1 week	4.00	70	4.3	7	30	298	505	48.0	81.3	40.9	8.7	2.0
						2.0	6		326	575	36.0	61.0	43.2	7.6	2.2
27	M	Cholecystitis with cholelithiasis	1 week	4.30	70	3.6	7		354	655	39.1	72.1	45.9	8.3	2.2
28	M	Chronic cholecystitis with cholelithiasis	12 days	4.16	70	6.0	10	30	687	1007	39.2	72.6	31.8	8.3	2.2
29	M	Stricture of common bile duct	5 weeks	4.32	72	5.8	11	30	421	712	33.5	60.2	41.0	7.5	2.3
30	M	Subacute cholecystitis, cholangitis, stone in common bile duct	4 months	4.10	65	2.2	10		521	805	43.0	77.0	34.0	9.1	2.3
31	M	Subacute cholecystitis, stone in common bile duct	3 years	3.67	63	5.6	7		479	807	37.3	67.8	40.6	8.4	
						3.5			307	650	23.5	45.0	52.7	8.7	2.1
32	M	Biliary cirrhosis with cholelithiasis	8 days	5.01	80	14.6	7	30	421	745	18.0	36.0	43.4		
33	M	Stricture of common bile duct	4 days	3.71	66	7.5	8		426	765	1.0	18	44.2		
34	M	Subacute cholecystitis with cholelithiasis, obstruction of common bile duct		5.02	80	2.4	9	30	321	547	43.0	74.1	41.2		
									359	680	26.5	51.1	47.1		
35	F	Stone in common bile duct	10 days	3.79	50	4.5	11	30	391	697	45.1	77.7	43.9	7.8	2.1
						2.4	8	30	452	765	49.1	81.8	40.8		
36	F	Stricture of common bile duct	11 months	4.00	65	5.5	8		332	575	46.3	80.6	42.2	7.9	2.1
						4.7	6		281	480	48.1	80.3	41.3		

\*van den Bergh reaction direct



TABLE II (Continued)

CASE	SEX	DIAGNOSIS	DURATION OF JAUNDICE	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG IN EACH 100 C C OF SERUM*	COAGULATION TIME		URIN, MG IN EACH 100 C C		SPEDIMENTATION RATE		HIFMATOCRIT		PROTEIN OF SERUM, GM	VISCOSITY OF SERUM
							MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MILLIMETERS	PER CENT	OXALATE, PER CENT	HEPARIN, PER CENT		
37	F	Stone in common bile duct	7 weeks	3.80	58	20.2	13		600	845	53.2	84.5	29.0	37.0	8.4	2.2
38	F	Stricture of common bile duct	2 weeks	2.92	59	3.0	9	30	1183	1650	57.0	87.0	28.0	34.5	8.4	2.1
39	F	Stone in common bile duct	8 weeks	2.77	50	9.0	8		742	990	60.0	91.0	25.0	34.0	5.9	1.8
40	F	Stone in common bile duct	10 weeks	3.52	65	4.9	8		510	785	53.0	84.0	35.0	37.0	7.0	2.1
41	F	Stricture of common bile duct	1 week	3.70	58	5.6	7	30	581	932	49.1	80.4	37.6	39.0	7.6	2.0
						5.1	7		546	862	50.8	83.2	36.0	39.0	7.5	2.1
42	F	Stricture of common bile duct	10 days	3.85	65	22.4	8	30	635	1115	52.0	80.0	43.0	44.0	9.0	2.6
						22.6	8	30				96.2	39.7		8.3	2.3
43	M	Intrahepatic jaundice	5 weeks	4.01	65	23.0	9	30	589	1007	54.3	86.1	11.5	37.0	8.2	2.1
44	F	Cirrhosis of liver	6 years	2.50	39	7.5	7	30	378	525	62.2	89.4	28.0	30.5	8.6	2.2
						7.6	10	30	408	585	63.0	89.7	30.2	29.8	7.8	2.3
45	F	Intrahepatic jaundice	3 weeks	3.71	65	37.5	8	30	243	397	42.6	69.4	38.8	38.7	8.5	2.3
46	F	Intrahepatic jaundice	1 year	3.75	45	3.0	7	30	675	945	52.0	79.5	28.5	34.6	8.5	2.3
						2.9	8		443	657	51.0	77.2	32.6	34.0	9.3	2.5

TABLE III  
CASES WITHOUT JAUNDICE

CASE	SEX	DIAGNOSIS	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG IN EACH 100 C C OF SERUM*	COAGULATION TIME		FIBRIN, MG IN EACH 100 C C		SEDIMENTATION RATE		HEMATOCRIT		BILIRUBIN OF SERUM, MG	VISCOSITY OF SERUM
						MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MAYER-FLERS	PER CENT	ONALALE, PER CENT	HIPATIN, PER CENT		
1	M	Normal						364	660						
2	M	Benign hypertension	5.20	78				242	450	11.8	22.6	46.1	48.0		
3	M	Thromboangitis obliterans						364	660			44.7	43.0		
4	M	Diabetes mellitus	4.50	70				304	580	14.3	26.4	47.5	46.0		
5	M	Anxiety neurosis	4.42	75				241	472	13.0	23.8	48.8	45.4		
6	M	Hypertension, arteriosclerosis	4.56	70				392	680	12.0	21.8	42.3	45.0	6.5	1.9
7	M	Arteriosclerosis, diabetes mellitus	5.15	75	1.7	7		326	635	10.5	21.6	48.6	51.5	7.7	2.0
8	M	Duodenal ulcer	4.98	70	2.4	8	10	295	420	2.5	4.6	46.2	46.0		
9	M	Phlebitis	3.99	65	2.2	8		282	537	17.9	33.7	47.5	47.0	6.9	1.9
10	M	Neurosis, pylorospasm, latent syphilis	5.09	82	2.3	7		282	532	5.5	10.5	46.9	48.0	8.0	2.0
11	M	Hyperinsulinism	4.96	78		6	20	417	790	39.8	73.0	47.2	45.5		
12	M	Gastric ulcer	4.56	80	1.1	6	30	319	575	12.0	21.8	44.4	45.0		
13	M	Duodenal ulcer	4.58	68	2.4	5		239	515	4.0	8.3	53.4	52.0	8.2	2.1
14	M	Thromboangitis obliterans	5.20	82	1.1	6	30	458	825	20.2	36.9	44.4	45.3		
15	M	Senile dementia	4.62	80	2.2	6		342	627	7.8	14.9	45.4	47.7		
16	M	Duodenal ulcer	4.40	62	0.8	11		173	287	6.0	10.0	39.5	40.0	7.2	1.8
17	F	Normal			2.3			223	415	10.0	18.5	46.2	46.0		
18	F	Nervous exhaustion	4.20	70	1.2	8		280	495	3.0	5.5	43.0	45.4		
				1.4	6	30		246	457	15.0	27.6	46.1	45.7		
19	F	Ventral hernia, chronic cholecystitis with cholelithiasis	4.69	80	1.7	8	30	361	697	29.2	58.0	48.1	49.7	8.7	2.5
20	F	Diabetes mellitus, chronic cholecystitis	5.11	75				318	505	26.0	41.9	37.0	38.0		
21	F	Chronic cholecystitis with cholelithiasis	4.28	60	0.3	8	30	419	685	36.5	62.9	38.7	42.0		
22	F	Leiomyoma of uterus, bilateral ovarian cystadenoma, chronic salpingitis	4.87	80	0.9	5		344	580	16.1	26.9	40.6	40.3		

\*Van den Bergh reaction indirect

In the cases in which the tendency to bleeding was demonstrated, the jaundice was usually of longer duration than in the other cases. Seventeen (85 per cent) of the former patients had jaundice of four weeks' duration or longer as compared with 14 (54 per cent) of the latter. This observation agrees with that of Petten, Walters, Judd and others.

The values for serum bilirubin were somewhat higher in the cases in which there was bleeding of some type. Of these, in 52.6 per cent the values for bilirubin in each 100 c c of serum were 15 mg or more, whereas, among the other cases of jaundice, similar values were found in only 26.9 per cent.

The coagulation time was found to be somewhat longer in cases in which there was a hemorrhagic tendency than in the other cases of jaundice. In 25 per cent of the former and in 77 per cent of the latter, the coagulation time was twelve minutes or more.

Values for blood fibrin were increased above normal in the greater number of cases of jaundice. The values were slightly higher in those cases in which there was no bleeding, as compared with those in which there was a tendency to bleeding.

Study of the sedimentation rate of erythrocytes revealed nothing significant. Four of our patients bled postoperatively and three of these had sedimentation rates higher than normal. However, the sedimentation rate was increased to more than normal in 41 other cases in which there was no postoperative bleeding (Tables I, II, III). This increase in the sedimentation rate is due in part to the effects of the increase in blood fibrin in many of the cases, and of the anemia which frequently occurs in jaundice.

Study of the erythrocyte count, percentage of hemoglobin and hematocrit readings in regard to the tendency to bleeding disclosed no significant relationship. Studies of the values for protein and viscosity of the serum were equally negative.

From our study, several interesting facts have been noted regarding the hemorrhagic tendency in jaundice. The content of blood fibrin could not be shown to be at fault in this condition. The values for blood fibrin in the cases in which there was a hemorrhagic tendency were slightly less than those in which there was no evidence of hemorrhage, but the majority were still higher than normal. Although Linton found the sedimentation test of prognostic value in regard to the possibility of bleeding, we should hesitate, from our results, to ascribe any importance to it. In considering the possibility of hemorrhage among jaundiced patients, the value for serum bilirubin and the duration of the jaundice must be considered, as well as the coagulation time.

#### CONCLUSIONS

Within the limits of the data presented, the deviations from the normal of the blood fibrin, sedimentation rate of erythrocytes, and protein and viscosity of the serum are not sufficiently great to be of significance in explaining the hemorrhagic tendency in jaundice. From the clinical standpoint the duration of the jaundice, its intensity as measured by the elevation in serum bilirubin and the prolongation of the coagulation time are of greater importance in judging the possibility of post-operative hemorrhage in the individual patient.

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## STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM\*

### XIX THE EFFECT OF DIET ON URINARY ACID AND AMMONIA EXCRETION IN MAN

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THE literature on ammonia metabolism is so large that one hesitates to present further data. However, there is surprisingly little in regard to normal ammonia excretion with diets of known acidity and with known nitrogen metabolism. This paper presents such data.

#### EXPERIMENTAL PROCEDURE AND PRECAUTIONS

The series of individuals (summarized in the addenda) studied in the research ward of the Massachusetts General Hospital were maintained on a continuously unvaried diet for periods of two weeks to three months. The routine methods used have been described in detail elsewhere.<sup>1</sup> Each patient was kept on a preliminary adjustment period of from three to six days; even so, a few observations have been excluded because it was obvious that a steady state had not been attained by the end of this time. Although the data here summarized comprise analyses (in duplicate) of over 400 twenty-four-hour specimens on nine patients, only the values attained at a steady state or "metabolic equilibrium" are given.

Successive specimens of urine from each subject were iced as soon as voided and kept, stoppered, with chloroform, until the end of each twenty-four-hour period, when titratable acidity (modified after Henderson and Palmer<sup>2</sup>) and ammonia (Folin<sup>3</sup>) were determined on the mixed twenty-four-hour specimen. Most of the ammonia analyses were made by the permittit-nesslerization method, but a considerable number were also made by the aeration procedure and satisfactory checks obtained.

In a few instances a relatively high ammonia concentration was encountered in an alkaline urine. It seemed desirable, therefore, to compare in an extreme case (patient DB) the summated acidity and ammonia content of individual specimens (analyzed as soon as passed) with the values later determined for the accumulated twenty-four-hour specimen. The results in Table I exclude the possibility of bacterial or chemical action (outside of the body) producing the high ammonia content and alkaline reaction, and justify the technique employed.

#### WHAT IS THE URINARY ACID AND AMMONIA EXCRETION OF A HUMAN ADULT ON A POTENTIALLY NEUTRAL DIET?

In Table II are given the figures for several patients, each value representing three days' excretion, i.e., the sum of three consecutive daily determinations. The

\*From the Medical Clinics of the Massachusetts General Hospital.

<sup>1</sup>Received for publication April 30, 1932.

<sup>†</sup>Alexander McPhedrin Research Fellowship in Clinical Medicine, University of Toronto.

TABLE I

PATIENT DB—TETANY, ACCOMPANYING STEATORRHEA  
ANALYSIS OF INDIVIDUAL AND COMBINED TWENTY-FOUR-HOUR SPECIMENS OF URINE

DAY EXCRETED	TIME EXCRETED	WHEN ANALYZED	VOLUME C C	ACID C C N/10	AMMONIA C C N/10
Wednesday	11 20 A.M.				
	9 00 A.M.	Wednesday Thursday	315	-25 -28	72 71
	1 00 P.M.	Wednesday	135	- 6	43
	7 30 P.M.	Wednesday	235	25	160
	10 00 P.M.	Wednesday	100	8	61
Thursday	12 30 A.M.	Thursday (1 00 A.M.)	555	15	85
	9 00 A.M.	Thursday Friday (5 00 P.M.)	470	-67 -63	124 124
Total Calculated Mixed Specimen		Thursday (10 00 A.M.)	1810	-49 -56	545 530

diets were planned to be potentially neutral according to data on foodstuffs used in this laboratory.\* In the table the potential acidity of the diet is also shown, when recalculated according to Sherman (1927).<sup>7</sup> The average ammonia excretion on such nearly neutral diets amounts to approximately 210 c.c. N/10 daily.

It is interesting to compare these values with those of Henderson and Palmer,<sup>8</sup> and with the results of Blatherwick.<sup>9</sup> The first of these investigators took no special precautions with regard to the potential acidity of the diet of their subjects, and the last used phenolphthalein as the reference indicator for titration. Their results, therefore, as recalculated in Table III for three day periods, tend to be higher than ours. We have no data, unfortunately, to enable us to calculate the actual acid-base content of Blatherwick's basal diet, which yielded nearly neutral urines and approximately the same titratable acidities as ours.

#### REPRESENTATIVE LEVELS OF URINARY ACID AND AMMONIA EXCRETION AT VARYING LEVELS OF ACID INTAKE

For purposes of clinical metabolic research, it was essential to establish standard levels of human urinary ammonia excretion at various levels of ingested acid. Such levels may be attained either by change in diet or by feeding alkali- or acid-producing electrolyte. The results shown in Fig. 1 demonstrate the marked rise (roughly logarithmic) in ammonia excretion with increasing potential acidity of the diet.

The actual equilibrium values involved are given in Table IV.

#### THE EFFECT OF ABSORPTION BY BOWEL

Variations of acid-base balance within the bowel may complicate the total acid-base balance, and particularly the urinary excretion. Salter, Fairquharson, and

\*The reliability of the extant data for calculating acidities of diets has been discussed elsewhere by Salter, Fulton, and Angier.<sup>4</sup>

TABLE II  
UPINAPY ACID EXCRETION (THREE DAY PERIODS†) ON "NEUTRAL DIET"

SUBJECT‡	TITRAT ABLE ACIDITY -CO <sub>2</sub> CC \ /10	AMMONIA CC \ /10	TOTAL ACID CC \ /10	POTENTIAL ACIDITY OF DIET CALCULATED FROM SHERMAN, 1927, CC \ /10	POTENTIAL ACIDITY OF DIET CALCULATED FROM DATA OF THIS LABORATORY CC \ /10 FOR 3 DAYS	NITROGEN BALANCE GRAMS PER 3 DAYS
W \	193 192	551 555	744 747	- 75	30	33 30
RN	531 416 526	692 598 635	1223 1014 1161	-141	24	-13 -05 -83
AN I*	-617 -635	1132 1201		-153	10	29 46
AN II*	95 - 62	637 526	732	-153	10	26 27
	196 255	724 660	920 915			-04 -32
DA	529 543 434	861 744 563	1390 1287 997	-129	4	-28 -46 38
ST	568 660 491	624 507 371	1192 1167 862	-129	4	45 10 67
LZ II	40 93	579 561	619 654	39	-120	0 -14
LZ XVIII*	-422 - 41 -268 -267	532 616 488 455		-165	- 26	81 63 100 80
DB*	-158 -400 156 -134	1732 1637 2030 1876	2186	-210	10	34 43 33 46
BE	318 177 257	445 362 468	763 539 725	-242	17	10 19 27
R F F	290 259	854 750	1144 1009	- 36	- 9	-17 09

†Figures given for three-day periods are the sums of three consecutive daily determinations

‡A brief description of each of these subjects is given in the addenda.

\*In alkaline urines the sum of titratable acidity and ammonia is omitted because of its questionable significance unless CO<sub>2</sub> is also measured

Tibbets<sup>3</sup> studied the neutralization of ingested sodium acid phosphate by bowel and kidney, respectively. They pointed out that the kidney could compensate for loss of base (through the bowel) by increase in ammonia production. This efficient base conservation by the kidney was shown by Gamble, Blackfan and Hamilton<sup>2</sup> to be the usual result of the administration of acid-producing salts (like calcium chloride). Such high ammonia excretion is, of course, usually accompanied by high urinary acidity. It cannot be assumed, however, that this is invariably true.

Of special interest is patient DB, whose minimal level of ammonia excretion was much higher than that of other patients of this series, even though the urine was frequently alkaline in reaction. This patient suffered intermittently from mild tetany due to deficient fat assimilation. It is unlikely, however, that tetany per se was responsible for the high ammonia excretion because a low ammonia was obtained with patient WR, who had parathyroid tetany of several years' standing.<sup>10</sup>

TABLE III  
URINARY ACIDITY VS EXCRETION OF TOTAL ACID  
(CALCULATED FOR THREE DAY PERIODS)

	TITRATABLE ACIDITY -CO CC \10	AMMONIA CC \10	TOTAL ACID CC \10
Henderson and Palmer <sup>6</sup> P <sub>H</sub> = 5.4 P <sub>H</sub> = 6.6	960 672	1101 1071	2061 1743
Blatherwick <sup>7</sup> Subject B	271 266 284 <u>821</u>	221 226 208 <u>655</u>	1476
Subject W	253 286 264 <u>803</u>	169 193 204 <u>566</u>	1369

TABLE IV  
MINIMAL AND MAXIMAL URINARY AMMONIA PRODUCTION (PER THREE DAYS)

SUBJECT	NEUTRAL DIET WITH ADDED NaHCO <sub>3</sub>		NEUTRAL DIET	HIGH ACID DIET		NEUTRAL DIET WITH ADDED NH <sub>4</sub> Cl	
	POTENTIAL ACIDITY OF DIET CC N/10	URINARY AMMONIA EXCRETION CC \10	URINARY AMMONIA EXCRETION CC \10	POTENTIAL ACIDITY OF DIET CC N/10	URINARY AMMONIA EXCRETION CC N/10	POTENTIAL ACIDITY OF DIET CC \10	URINARY AMMONIA EXCRETION CC N/10
DA	-2103 -7622	242 293	563 511	2111 2170	1389 1359	6736 6736	3948 4787
ST	-2138 -7304	100 73	507 371	2171 2171	1227 1433	6736 6736	4737 6131
AN I	-4715 -5190	170 142	1132 1201	2069 2069	2136 2549	4142 3376	3834 3982
DB*	-4532 -4615	846 785	2030 1876			3366 3366	4470 4438
WR†			728				

\*Tetany, accompanying steatorrhea

†Tetany, idiopathic presumably hypoparathyroidism



It is quite possible, on the other hand, that the steatorrhea in this case was directly responsible for the high urinary ammonia. The subject lost an unusual amount of fixed base (in the form of soap) by feces, and was presumably forced to make extraordinary demands upon devices which conserve fixed base, e g, ammonia-production.

The mechanism at work here is presumably quite similar to that of the acid-producing salts. The essential differences are first, that the acid involved is an organic acid (derived from neutral fat), and second, that there is no rise in urinary acid because there is no exogenous acid radicle to be excreted. That such excessive loss of fixed base may produce a "relative acidosis" has been shown by several investigators, whose work has been summarized by Shohl.<sup>11</sup>

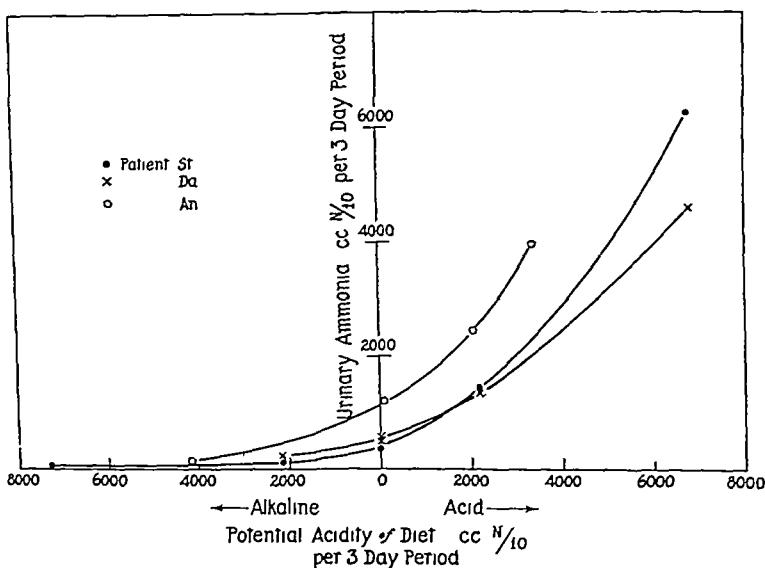


Fig. 1—Urinary ammonia increases when potential acidity of the diet is increased

In Table V is contrasted the fixed base excretion of patient DB with that of two normal young adults. The titratable alkalinity\* of the fecal ash is also shown. Patient DB's fixed base excretion (by bowel) is high, and the titratable ash over 50 per cent of it. When calcium chloride is added to the diet, the situation is further exaggerated because calcium is held in the bowel by excess of fatty acid. In the latter instance, titratable ash approximately equals total base.

The high urinary ammonia output in case DB may be regarded as pathologic but other similar figures were obtained with subject AN (see Table II, AN I), who when taking a potentially neutral diet, excreted considerable amounts of ammonia in an alkaline urine. It is noteworthy that the feeding of large amounts of acid-producing substances (including ammonium chloride) to this subject resulted in so great an increase in ammonia excretion that the urine remained alkaline even under the stress of eliminating a great excess of acid radicles.<sup>12</sup> Curiously enough, after a vacation of six months, this patient returned to the former ward and identical diet, but failed to excrete an alkaline urine (see Table II, AN II). Such excre-

\*Titratable ash was determined by titrating the dry ash with HCl to methyl red

TABLE V  
RELATION OF URINARY ACID EXCRETION TO INORGANIC CONSTITUENTS OF FECES  
(THREE DAY PERIODS)

SUBJECT	DIET	URINARY			TOTAL FIXED BASE OF INGESTA CC N/10	FECAL	
		TITRATABLE ACIDITY - CO. CC N/10	AMMONIA CC N/10	TOTAL ACID CC N/10		TOTAL FIXED BASE CC N/10	TITRAT ABLE ASH CC N/10
AD Normal	Control	384	835	1219	5142	1000	296
	Control	300	836	1136	5142	670	382
	Added NaCl	337	922	1259	10272	760	392
	Added NaCl	383	984	1367	11982	765	386
	Added NaCl	111	1186	1297	12257	690	382
CW Normal	Control	584	983	1567	5142	930	382
	Control	570	951	1521	5142	1120	385
	Added NaCl	421	1007	1428	10275	940	368
	Added NaCl	536	990	1411	11939	1060	423
	Added NaCl	353	1174	1427	12840	870	290
DB Tetany, Accompanying Steatorrhea	Control	65	2065	2130	5175	2530	1380
	Added CaCl <sub>2</sub>	401	3543	3944	10035	3980	4250

tion values serve to emphasize our lack of knowledge concerning ammonia excretion in health. They present no explanation for the preferential conservation of base by ammonia-production rather than by titratable acidity.

#### LAG IN URINARY EXCRETION FOLLOWING CHANGE IN DIET

Peters and Van Slyke<sup>13</sup> have commented upon the sluggish response of ammonia excretion to the ingestion of acid. In order to determine the length of time which must elapse following a dietary change before a steady metabolic state is attained, daily determinations were made until constant excretion values were approached. From a survey of Fig. 2 and similar data obtained from the subjects listed in Table II it seems obvious that equilibrium values may not be reached in less than five to seven days after a dietary change. It is true, however, that the excretion of urinary titratable acidity reaches equilibrium level earlier than other factors, such as calcium and ammonia excretion.

Fig. 2 indicates, also, how constant the acid-excretion of a single individual may become on a constantly continued metabolic regimen when once a plateau-level of excretion has been attained.

#### EFFECT OF NITROGEN BALANCE ON URINARY ACID EXCRETION

From inspection of the nitrogen balances listed in Table II, it appears that the subjects studied were for the most part in nitrogen equilibrium, or retained a surplus of nitrogen. The chief exception is subject RN, who in one period apparently used 8 gm of body nitrogen. Estimating the potential acidity of one gram of human protein-nitrogen as 44 cc of N/10 acid (see data for meat given by Sher-

man\*) this negative nitrogen balance might conceivably account for 366 c.c. of N/10 acid during the three days in question. As one surveys the apparently spontaneous variations which occur in total acid excretion, one is led to believe that the excretions given in Table II may be regarded as essentially independent of the nitrogen balance.

This impression is substantiated, if one studies specifically the effect of two common acid-producing agents, i.e., protein and ammonium chloride. As a crucial test, we studied the effect of these substances on ammonia excretion when sufficient sodium bicarbonate was fed (simultaneously) to balance their potential acidity.

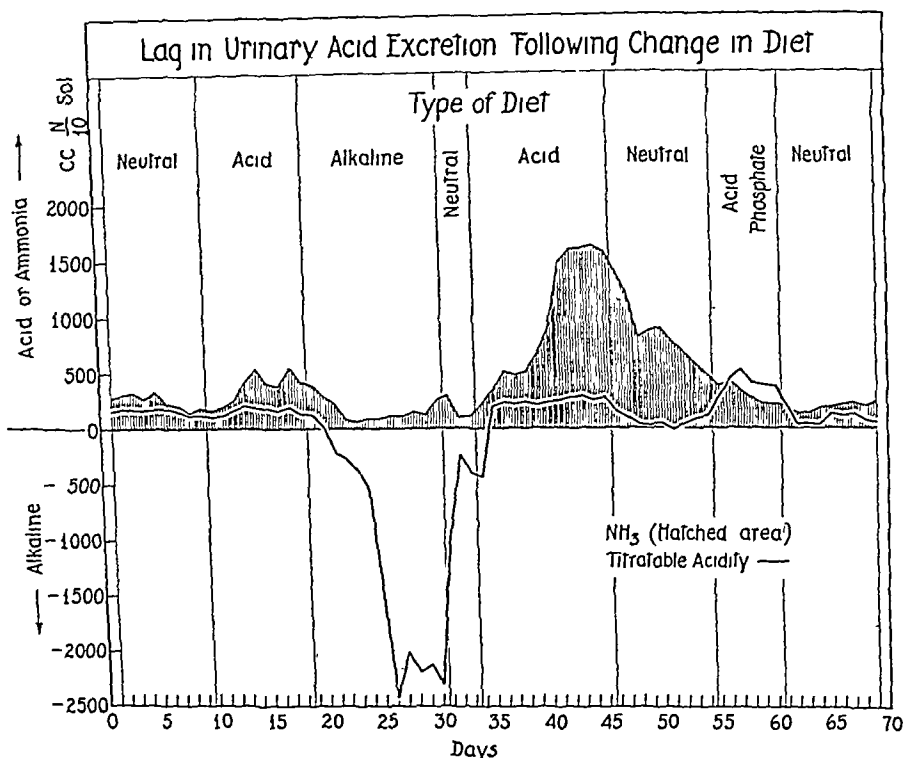


Fig. 2.—Excretion of acid in consecutive twenty-four-hour specimens of urine during variation in the potential acidity of the diet. The term diet is here used to designate all ingesta including both food and medication. The daily potential acidity of the respective diets indicated in the figure are (in sequence)

Neutral = 0    Acid = 724 c.c. N/10    Alkaline = 714 c.c. N/10 for the first 6 days 2600 c.c. N/10 thereafter

Neutral = 0    Acid = 738 c.c. N/10 for the first 5 days 2266 c.c. N/10 thereafter

Neutral = 0    Acid phosphate = 713 c.c. N/10

(For description of subject see DA in addenda.)

#### THE EFFECT OF INGESTED NEUTRALIZED PROTEIN ON AMMONIA EXCRETION

In order to determine whether the ingestion of large amounts of protein per se affected the formation of urinary ammonia, a normal adult was fed protein to the extent of 202 gm. daily after a control test on a neutral diet containing 58.6 gm. of protein per day. Simultaneously with the high-protein diet sufficient  $\text{NaHCO}_3$  was fed to hold the titratable acidity at its former level. Under these circumstances, only a slight (possibly negligible) rise in ammonia was observed. When alkali was

discontinued, a marked increase in urinary ammonia (as well as titratable acidity) occurred (see Table VI). These results indicate that the effect of a high-protein diet in increasing urinary ammonia is due mainly to its acid catabolites. They show that when such protein is neutralized the resulting ammonia excretion progressively approaches a low plateau level. They minimize the importance, therefore, both of the nitrogen balance, when positive, and of the urinary nitrogen excretion (*per se*) as prime factors in determining urinary ammonia.

TABLE VI

EFFECT OF INGESTED PROTEIN ON URINARY AMMONIA PRODUCTION  
(THREE DAY PERIODS)

SUBJECT R F F

	POTENTIAL ACIDITY OF DIET cc N/10	NaHCO <sub>3</sub> ADDED GRAMS	POTENTIAL ACIDITY OF TOTAL INGESTA cc N/10	URINARY	
				TITRATABLE ACIDITY - CO <sub>2</sub> cc N/10	AMMONIA cc N/10
Low Protein, Neutral Diet	-9		-9	321 290 259	934 854 750
High Protein Diet, Neutralized by NaHCO <sub>3</sub>	3387	27 26	233 352	-27 50	928 1088
High Protein Diet, plus Excess NaHCO <sub>3</sub>	3387	90	-7323	-7259	226
High Protein Diet, without NaHCO <sub>3</sub>	3387		3387	1568	2240

TABLE VII

EFFECT OF INGESTED AMMONIA ION ON URINARY AMMONIA  
(THREE DAY PERIODS)

SUBJECT AN II

	POTENTIAL ACIDITY OF DIET cc N/10	NaHCO <sub>3</sub> ADDED GRAMS	POTENTIAL ACIDITY OF TOTAL INGESTA cc N/10	TITRATABLE ACIDITY - CO <sub>2</sub> cc N/10	AMMONIA cc N/10
Control, Neutral diet	-7 10			95 - 62	638 526
Neutral Diet plus NH <sub>4</sub> Cl (6 grams daily)	10		3334 3376	702 654	1953 2637
Neutral Diet plus NH <sub>4</sub> Cl (6 grams daily) plus NaHCO <sub>3</sub>	10	37 29 23	-511 -135 698	-683 -297 286	492 558 613

## AMMONIA INGESTION VS AMMONIA EXCRETION

The possibility that ingested NH<sub>4</sub> ion (administered in the form of NH<sub>4</sub>Cl) might contribute to urinary ammonia has been commented upon by Gamble, Blackfan, and Hamilton.<sup>9</sup> In order to control our neutralized protein experiment, sub-

ject AN, after attaining equilibrium on a nearly neutral diet, was given 6 gm of  $\text{NH}_4\text{Cl}$  daily. Subsequently,  $\text{NaHCO}_3$  was also given (alternately with each dose of  $\text{NH}_4\text{Cl}$ ) in sufficient quantity to produce a nearly neutral urine. The results are in agreement with a similar experiment of Haskins<sup>14</sup> who fed ammonium carbonate directly.

## THE INFLUENCE OF PHOSPHATE EXCRETION ON URINARY AMMONIA

The reason for the increase in urinary ammonia following the ingestion of un-neutralized protein is not clear. One might on first thought suspect the increased elimination of phosphoric acid (from oxidized protein) as the causative agent. The influence of acid phosphate on the elimination of ammonia in the urine has been discussed by Marriott and Howland,<sup>15</sup> however, who pointed out that increase in

TABLE VIII  
EFFECT OF PHOSPHATE INGESTION ON URINARY AMMONIA AND TITRATABLE ACIDITY  
(THREE-DAY PERIODS)

SUBJECT	DIET	PHOSPHORUS IN FOOD GM	TOTAL PHOSPHORUS INGESTED GM	POTENTIAL ACIDITY OF INGESTA CC N/10	TITRATABLE ACIDITY-CO <sub>2</sub> CC N/10	AMMONIA CC N/10	TOTAL ACID CC N/10
DA	Neutral control diet (moderate phosphorus)	2.2 2.2		4 4	543 434	744 563	1287 997
	Acid diet	2.8 2.9		2111 2170	585 508	1389 1359	1974 1867
	Neutral diet plus $\text{NaH}_2\text{PO}_4$	2.2	10.1	2142	1179	699	1878
WN	Neutral control diet	1.9 1.9		31	193 192	551 555	744 747
	Control diet plus $\text{NaH}_2\text{PO}_4$	1.9 1.9	10.8 10.8	2428 3235	1222 870	757 776	1979 1646
	Control diet immediately after acid phosphate	1.3 1.9 1.9		102 - 18 31	381 141 362	956 914 932	1337 1055 1294
LZ	Neutral diet, moderate phosphorus	2.1 2.1		- 66 - 83	-121 - 4	608 608	
	High equimolecular phosphate	2.1 2.1	5.4 5.4 5.4	268 324 268	-263 114 70	541 525 362	639 432
	Control diet	1.8 1.8 1.2		24 24 - 13	531 416 526	692 598 635	1223 1014 1161
	Control diet plus $\text{Na}_2\text{HPO}_4$	1.8 1.8 1.8	8.5 8.7 8.4	-284 -348 -328	70 -397 17	306 313 276	376 293
	Control diet	1.6 1.9		- 96 - 8	305 180	416 428	721 608
	Control diet plus $\text{NaH}_2\text{PO}_4$	1.9 1.9	9.0 8.9	1962 2025	1008 878	697 573	1705 1451

phosphate excretion spares ammonia-production. Similarly, Haldane<sup>10</sup> found that ingestion of 30 gm. of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  produced no fall in alveolar  $\text{CO}_2$ , nor rise in urinary ammonia although the acidity of the urine rose to 0.114N. This effect is not due to any peculiar suppressing influence of the phosphate radicle per se upon ammonia production, but rather is a logical result of the relation of urinary phosphate to acid excretion by the kidney.<sup>8</sup>

In Table VIII are presented data on total acid excretion by individuals who were fed acid or alkaline phosphate. The results indicate very little change in ammonia excretion despite marked variation in excretion of titratable acid. They substantiate, therefore, the findings of previous investigators, and are presented only because they represent quantitative excretion-levels in human beings on known intakes. They point to other acidic groups, e. g., sulphur, in protein as the cause of increased ammonia excretion.

#### SUMMARY

In human adults on a potentially neutral diet, urinary ammonia amounts to about 210 c. c. N/10 daily.

When the need for acid elimination is controlled (by adjusting the potential acidity of the food metabolized) the urinary ammonia excretion of human beings is found to increase in a roughly logarithmic fashion as more acid is fed.

Without recourse to inorganic acids (or acid-producing salts), it is difficult to attain a normal ammonia excretion above 800 c. c. N/10 daily, because the phosphate in most acid-producing foods tends to relieve urinary ammonia production.

The urinary response to acid or basic diets is complicated by (1) variations in base excretion by the gastrointestinal tract and (2) by lags in the renal base-conserving mechanisms (i. e., ammonia and titratable acidity).

High protein intake does not increase ammonia production, provided that simultaneously enough alkali is administered to neutralize its acid catabolites.

#### ADDENDA

##### DESCRIPTION OF PATIENTS

A brief description of each of the patients (listed in Table III) used in this investigation follows.

WN. A married female of thirty-four years of age, weighing 89 kilos, suffering from chronic atrophic arthritis of two years' duration.

RN. A single female of thirty-nine years of age, weighing 56 kilos, suffering from rheumatic heart disease (mitral stenosis), chronic bronchitis, and bronchial asthma. During the period of observation she had no cough, her chest was clear and there were no signs of myocardial failure.

AN. A male, aged sixteen, weighing 34 kilos, suffering from marked structural scoliosis of spine. Admission I—at rest in supporting plaster shell. Admission II—six months after a spinal fusion for correction of his deformity, followed by a vacation.

DA. A male aged thirty-seven, weighing 58 kilos, recovering from lead poisoning. Mild extensor paresis of upper extremities (fingers and wrists). No colic during periods presented here.

ST A male, aged forty-six, weighing 51 kilos, recovering from lead poisoning  
No paralysis No colic during periods presented here

LZ A male, aged eighteen, weighing 60 kilos, recovering from chronic multiple neuritis, confined to bed because of weakness of extremities, but feeling well

DB A female, aged twenty-seven, weighing 43 kilos, suffering from tetany associated with deranged fat absorption for some four years Free from tetany at time of this investigation, but serum calcium was only 5.6 mg per 100 c c

BE A male, aged fifty-eight, weighing 55 kilos, suffering from chronic sciatic neuritis of ten months' duration

RFF A male, aged thirty, weighing 78 kilos, healthy, normal, a physician

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## VARIATIONS IN CERTAIN CONSTITUENTS OF THE BLOOD OF RELATIVELY NORMAL INDIVIDUALS\*†

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THIS study was undertaken because of the lack of agreement among authors in regard to the composition of the blood. Attention will be directed to two constituents only, although equally great discrepancies are found in many others. A comprehensive review will not be attempted.

Hammett (1920) in sixty determinations on nine individuals, found a range of 27.3 to 45.5, with an average of 35.6 mg per cent for total nonprotein nitrogen, for urea nitrogen, he found the limits to be 9.7 and 23.8, with an average of 17.1 mg per cent. In his series, the urea nitrogen comprised 47.8 per cent of the total nonprotein nitrogen.

Berglund (1922) studied twelve normal subjects, in whom he found a range of 27.8 to 39.4, with an average of 32.1 mg per cent, the urea nitrogen limits were 8.9 and 15.2, with an average of 11.5 mg per cent, in this series, the urea nitrogen formed 35.82 per cent of the total. This author stated that in wealthy subjects, on a high protein diet, he found the total nonprotein nitrogen to be between 40 and 45 mg per cent.

Myers (1924) gave the range of total nonprotein nitrogen as 25 to 35 and of urea, 12 to 15 mg per cent, he states that the urea nitrogen forms 50 per cent of the total nonprotein nitrogen.

Mathews (1925) gave the range as 26 to 43 for total nonprotein nitrogen, and 10 to 22 for urea nitrogen.

Hawk and Bergeim (1927) gave 25 to 30 mg per cent for the total, and stated that the urea nitrogen forms 50 per cent of the total nonprotein nitrogen.

Bodansky (1930) gave the range as 30 to 35 for total nonprotein nitrogen, and 14 to 20 for urea nitrogen, and stated that the urea nitrogen forms 50 to 60 per cent of the total.

In view of such discrepancies, it was decided to study several constituents of the blood in a large group of men and women. The individuals who volunteered to act as subjects were members of the faculty, students, and parents or relatives of students. All subjects were in comparatively good health, engaged in various types of work, and free from known disease, consequently, they were considered relatively normal.

The group comprised 105 men and 109 women. The mean age of the men was 25.65 years, of the women, 29.27 years, and of the group as a whole, 27.49 years. The age range for the men was 18 to 63 years, and for the women 18 to 77 years.

The blood (20 cc) was drawn by venipuncture between seven and eight o'clock in the morning, after a twelve to fourteen hour fast. Only those cases in

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which a complete analysis of the seven selected constituents was accomplished were considered in the statistical treatment of the results

The following seven constituents were determined, by the methods designated

- 1 True glucose Shaffer Somogyi (1929)
- 2 Serum calcium in duplicate Clark Collip (1925) modification of Kramer Tisdall (1921)
- 3 Inorganic phosphate Fiske Subbrow (1925)
- 4 Serum chloride Whitehorn (1920-21)
- 5 Urea nitrogen direct Nesslerization of the unaltered blood filtrate, Folin (1930 b), after incubation of the buffered whole blood with urease paper, Folin (1930 a)
- 6 Total nonprotein nitrogen Koch McMeekin (1924) oxidation and direct Nesslerization of the tungstate sulphuric acid filtrate, Folin (1919)
- 7 Uric acid Benedict (1922)

Table I presents the means found for the men, women, and group as a whole, and the range of each constituent

TABLE I

CONSTITUENT	MEN	MEANS (MG PER CENT)		RANGE COMBINED
		WOMEN	COMBINED	
Sugar	83.95 $\pm$ 0.47	84.07 $\pm$ 0.45	84.01 $\pm$ 0.33	59.65 - 101.59
Calcium	10.581 $\pm$ 0.020	10.601 $\pm$ 0.026	10.591 $\pm$ 0.016	9.69 - 12.15
Inorganic phosphate	3.433 $\pm$ 0.027	3.406 $\pm$ 0.029	3.419 $\pm$ 0.020	2.58 - 4.90
Sodium chloride	574.4 $\pm$ 1.0	579.6 $\pm$ 1.0	577.1 $\pm$ 0.72	535 - 609
Urea nitrogen	13.56 $\pm$ 0.19	13.49 $\pm$ 0.19	13.53 $\pm$ 0.13	7.5 - 23.8
Total nonprotein nitrogen	34.87 $\pm$ 0.23	32.98 $\pm$ 0.26	33.91 $\pm$ 0.18	21.3 - 44.1
Uric acid	3.417 $\pm$ 0.034	3.070 $\pm$ 0.029	3.240 $\pm$ 0.024	2.10 - 5.52

The urea nitrogen formed 39.90 per cent of the total nonprotein nitrogen

Table II presents the standard deviations of each constituent

TABLE II

CONSTITUENT	STANDARD DEVIATIONS		
	MEN	WOMEN	COMBINED
Sugar	7.18 $\pm$ 0.33	6.99 $\pm$ 0.32	7.08 $\pm$ 0.23
Calcium	0.305 $\pm$ 0.014	0.399 $\pm$ 0.018	0.356 $\pm$ 0.012
Inorganic phosphate	0.415 $\pm$ 0.019	0.449 $\pm$ 0.020	0.433 $\pm$ 0.014
Sodium chloride	15.63 $\pm$ 0.73	15.71 $\pm$ 0.72	15.65 $\pm$ 0.51
Urea nitrogen	2.84 $\pm$ 0.13	2.93 $\pm$ 0.13	2.884 $\pm$ 0.094
Total nonprotein nitrogen	3.57 $\pm$ 0.17	4.05 $\pm$ 0.18	3.94 $\pm$ 0.13
Uric acid	0.522 $\pm$ 0.024	0.456 $\pm$ 0.021	0.520 $\pm$ 0.017

Table III presents the coefficients of variation of each constituent

TABLE III

CONSTITUENT	COEFFICIENTS OF VARIATION		
	MEN	WOMEN	COMBINED
Sugar	8.55	8.31	8.43
Calcium	2.88	3.76	3.36
Inorganic phosphate	12.09	13.18	12.67
Sodium chloride	2.72	2.71	2.71
Urea nitrogen	20.92	21.71	21.32
Nonprotein nitrogen	10.23	12.28	11.61
Uric acid	15.28	14.87	16.04

The differences between the means for the men and women are given in Table IV. The means for the women are used as the subtrahends.

TABLE IV

CONSTITUENT	DIFFERENCES BETWEEN MEN AND WOMEN	
	DIFFERENCE	DIFFERENCE/ERROR
Sugar	$-0.12 \pm 0.65$	0.18
Calcium	$-0.020 \pm 0.033$	0.61
Inorganic phosphate	$+0.027 \pm 0.039$	0.69
Sodium chloride	$-5.2 \pm 1.4$	3.7
Urea nitrogen	$+0.07 \pm 0.27$	0.26
Total nonprotein nitrogen	$+1.89 \pm 0.35$	5.4
Uric acid	$+0.347 \pm 0.045$	7.7

We were interested in determining whether or not a relationship existed between certain pairs of constituents. This was studied by means of the correlation coefficients. The results are presented in Table V.

TABLE V

CORRELATION COEFFICIENTS	
Urea—total nitrogen (nonprotein)	$+0.542 \pm 0.032$
Sugar—phosphate	$-0.089 \pm 0.046$
Sugar—calcium	$+0.045 \pm 0.046$
Calcium—phosphate	$+0.134 \pm 0.045$

The relationship between urea nitrogen and total nonprotein nitrogen is the only one of significance.

#### SUMMARY AND CONCLUSIONS

Seven constituents of the blood have been studied on 214 (105 men and 109 women) relatively normal individuals in a fasting condition.

The age range was eighteen to seventy-seven years with an average of 27.49 years.

The mean for true glucose is  $84.01 \pm 0.33$ , this agrees well with previous work by this method (Holt and Graisheimer, 1931, and Glassberg, 1931). The difference between the sexes is not significant. The true glucose level bears no relation to the level of either calcium or inorganic phosphate, this is shown by the low correlation coefficients.

The mean serum calcium is  $10.591 \pm 0.016$ , the difference between the sexes is not significant. The present values are slightly lower than those found earlier (Graisheimer, 1929), but the difference between the two series is of doubtful significance.

The mean inorganic phosphate is  $3.419 \pm 0.020$ , with no significant difference between the sexes. The inorganic phosphate level bears no relation to the serum calcium level, as shown by the absence of correlation.

The mean serum sodium chloride is  $577.06 \pm 0.72$ , with no significant difference between the sexes.

The mean urea nitrogen is  $13.53 \pm 0.13$ , with no significant difference between the sexes, it formed 39.90 per cent of the total nonprotein nitrogen, it bears a defi-

rate relation to the total, as would be expected, and as is shown by the high degree of correlation

The total nonprotein nitrogen is  $33.91 \pm 0.18$ , the mean for the men is significantly higher than that for the women

The mean uric acid is  $3.240 \pm 0.024$ , the mean for the men is significantly higher than that for the women

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## STUDIES IN THE SEROLOGY OF SYPHILIS\*

### XI A CLINICAL AND STATISTICAL EVALUATION OF A NEW FLOCCULATION TEST FOR SYPHILIS BASED ON 26,611 TESTS IN COMPARISON WITH THE WASSERMANN REACTION, AND 2473 IN COMPARISON WITH THE KAHN

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PRECIPITATION tests for syphilis are handicapped by two inherent difficulties (1) the large personal factor involved in the reading of weak positive reactions, in which the aggregates are difficult to detect and (2) the multiplicity of variables which must be rigidly controlled in order to obtain consistent or even reliable results, variables which have been discussed in detail elsewhere † These two difficulties are masked by the apparent technical simplicity of the reaction, and only come to light when, upon analyzing the results in comparison with the Wassermann reaction, one uncovers a high incidence of false positive or false negative results

In an attempt to eliminate, or at least decrease the importance of these two disturbing factors, the author recently devised a precipitation test,\*\* embodying (1) an optimum adjustment of the variables which control the aggregation, insofar as these could be ascertained on empirical and theoretical grounds (2) the use of the centrifuge to facilitate aggregation, and (3) the use of a new sensitizing substance, corn germ sterol, as an adjunct to cholesterol The advantages of the test were stated to be (1) a greater sensitivity (2) simplicity of technique, as no titrations are necessary, and the test involves the use of only one tube, (3) an increased reliability, due to the ease of reading results, and the wide margin of safety in the adjustment of the reagents (4) convenience, as the antigen dilution keeps for at least three days, and the incubation time can be varied from one-half hour, for emergency tests, to as long as twenty-four hours, for maximal sensitivity, with four hours as the routine incubation period, (5) cheapness, one cubic centimeter of antigen sufficing for 50 to 100 tests

The present paper is an analysis of the results obtained with the test at the Johns Hopkins Hospital during the period August 10, 1931, to April 1, 1932 They are presented as statistical evidence for the sensitivity and reliability of the reaction as claimed in the first communication The material comprises 26,611 sera of a general dispensary and hospital population, divided into two groups The one group consists of 10,383 tests on approximately 1500 known syphilitic patients under active treatment or observation in the Syphilis Clinic of the Johns Hopkins Hospital The remaining 16,228 tests were on approximately 14,000 patients in the remainder of the dispensary and in the general hospital This group included 2441 tests on children under fourteen years of age, some of whom were under treatment for congenital syphilis

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Every serum was tested by a four hour ice box Wassermann reaction and by the precipitation test in question. In addition, 1328 sera from Group 1 (syphilitic), and 1045 sera from Group 2 (general hospital and dispensary) were tested by the Kahn reaction. The same individual who read the Wassermann results (a laboratory technician) also read the precipitation reactions. Kahn test results were read by the author.

### I *Technic of the Various Tests*—

1 *The New Precipitation Test—Antigen* Fifty grams of dried powdered beef heart (Difco) are extracted at 37° C for fifteen minutes with 200 c c of ether with intermittent shaking. The process is repeated three times and the ether filtrates discarded. The powder is then dried and extracted with 250 c c of 95 per cent ethyl alcohol for three to five days at 37° with repeated shaking. The extract is then filtered and the moist powder washed with 3 to 50 c c portions of 95 per cent alcohol. The combined filtrate and washings are evaporated down to 250 c c. One and a half grams cholesterol and 1.5 grams corn germ sterol are then added and dissolved by boiling, a final concentration of 0.6 per cent for each. The corn germ sterol can be obtained from the Digestive Ferments Company, Detroit.

*Technic of Test* The antigen is heated at 55° to 75° C for a few minutes to dissolve all of the sensitizer. One and three-tenths volumes of 4 per cent NaCl are blown into 1 volume of antigen, and the milky suspension allowed to "ripen" for at least thirty minutes at room temperature. Kept in the ice box, this suspension is good for forty-eight to seventy-two hours, gradually increasing in sensitivity. Our routine procedure was to prepare the dilution twenty-four hours in advance. This routine "aging" has since been prolonged to forty-eight hours.

The sera to be tested are inactivated at 56° C for twenty minutes. To 0.4 c c are added 0.04 c c of the antigen dilution, the tubes shaken for two minutes, and placed at 37° C for four hours. At the end of this time, the tube is centrifuged for fifteen minutes at 1500 revolutions per minute, and 1.2 c c of 0.85 per cent NaCl then added.

In a strongly positive serum, there is seen a very coarse aggregation of the antigen with a perfectly surrounding clear fluid. In a less strongly positive serum there are definite aggregates. A negative serum remains opalescent, with a homogeneous cloud of refractile particles. Any intermediate result, in which the contents of the tube seem finely granular, lacking the watered-silk appearance of the negative reaction, or in which there are a few nondescript particles, is read as doubtful. All such doubtful results are recentrifuged at higher speed after shaking, if the test is really positive, the antigen forms a coherent white flake at the bottom of the tube. The re-centrifugation of any doubtful result is essential for best results.

Reports are made as positive, negative, or doubtful.

2 *Wassermann Reaction—Antigen\** Fifty grams of dried powdered beef heart (Difco) are extracted with 250 c c of ether for fifteen minutes at 37° C. The ether filtrate is discarded. The moist powder is washed on the filter with 100 c c

\* As will be shown in a later paper it has since been found that the precipitation antigen can be used in the Wassermann test as well, giving identically the same results as the Wassermann antigen used in this series. For use in the Wassermann test the precipitation antigen is diluted 1:100 prepared by dropping two volumes of salt solution slowly into one volume of antigen and then pouring in the remaining 97 volumes of salt solution.

of warm ether, dried, and extracted at 37° C for four days with 250 c c of 95 per cent ethyl alcohol. After filtration, the moist powder is washed with 100 c c of alcohol and the combined alcoholic filtrate and washings evaporated down to 75 c c. Seventy-five cubic centimeters of absolute alcohol are then added. The extract is then sensitized with 0.8 per cent cholesterol and 0.6 per cent sitosterol (8 and 6 mg per c c respectively), which are dissolved by boiling. The sitosterol can be obtained from the Digestive Ferments Company, Detroit.

*Technic of Test* The clear serum is inactivated at 56° C for twenty minutes, and 0.1, 0.1, and 0.05 c c respectively placed in three tubes. Complement is prepared by pooling the blood of at least 4 guinea pigs, allowing it to clot overnight at ice box temperature, and centrifuging. Two-tenths cubic centimeter of a 1:12 dilution in 0.85 per cent NaCl are added to all three tubes.

The antigen is heated at 56° C for a few minutes to redissolve excess sensitizer, and diluted by slowly dropping one volume of antigen, with shaking, into 200 volumes of 0.85 per cent salt solution\*. Two tenths cubic centimeter of the dilution are added to Tubes 2 and 3, Tube 1 serving as an anticomplementary control.

Two-tenths cubic centimeters of salt solution are added to Tube 1, making a total volume of 0.5, 0.5, 0.45, in the three tubes.

The tests are then placed in the ice box for four hours, followed by one-half hour incubation at 37° C. Four-tenths cubic centimeters of a sensitized cell suspension are then added and the results read after fifteen to twenty minutes at 37° C.

The sensitized cells are prepared by washing citrated sheep's blood with 10 volumes of salt, and diluting the measured packed cells with 32 volumes of salt solution. The minimal dilution of amboceptor which will cause the hemolysis of this suspension in one-half hour is then determined (0.2 c c of amboceptor plus 0.2 c c per cent cells plus 0.2 c c 1:12 complement plus 0.4 c c salt solution). The 3 per cent cell suspension is then sensitized with an equal volume of amboceptor dilution containing 2½ to 3 times this minimal hemolytic quantity. Four-tenths cubic centimeter of the resultant 1½ per cent suspension are used in the test.

Reports are made as positive, negative or doubtful.

3 *Kahn Reaction*—Antigen was obtained from Dr. R. L. Kahn, to whom I express my appreciation. The technique used is exactly that described in his book on the subject, except that only one tube was used with a 1:8 antigen serum ratio (0.4 serum + 0.05 c c of antigen dilution).

## II *Sensitivity Results in a Known Syphilitic Population (10,383 Tests)*—

a The results summarized in Tables I and II are self-explanatory. From 72 to 75 per cent of the sera in a general syphilitic population under active antisyphilitic treatment or observation, gave positive or doubtful precipitation tests as compared with 58.7 per cent for the Wassermann† and 60.2 per cent for the Kahn reactions. Even these figures do not indicate the degree of greater sensitivity, for of those detected by the Wassermann and Kahn, 7.7 per cent and 6.2 per cent respectively were doubtful, while a definite reading of positive or negative was made

\*A more sensitive antigen dilution can be prepared by dropping 2 volumes of salt solution, slowly and with shaking into 1 volume of antigen and after a two- to five minute interval, adding the remaining 193 volumes of salt solution.

†A systematic technical error (the use of unsterile citrate in the collecting of the sheep's blood) caused the cell suspension to be quite fragile over a period of at least three months. Due to this error, the figures given for the positive Wassermann reactions are at least 5 per cent too low, as will appear in a forthcoming analysis of a second series of 30,000 tests.

in all except 1.6 per cent of the precipitation tests. The large number of anticomplementary sera in the Wassermann reaction in this group was found to be due to the presence in the serum of arsenicals inadvertently introduced in the collecting tube, such specimens being particularly likely to be anticomplementary when allowed to stand at room temperature over the week-end.

The greater sensitivity of the new precipitation test over the Wassermann and Kahn reactions (and we wish to emphasize the fact that this is a comparatively sensitive Wassermann technique), becomes clearly manifest when we note that the precipitation test detected 15.3 per cent (1594 sera) missed by the Wassermann reaction entirely and 13.4 per cent missed by the Kahn while the reverse was true in only 1.1 and 1 per cent respectively.

TABLE I

COMPARISON OF THE WASSERMANN AND PRECIPITATION TESTS IN 10,383 SERA FROM A SYPHILITIC POPULATION UNDER TREATMENT OR OBSERVATION

	ABSOLUTE AGREEMENT 72.5%			RELATIVE AGREEMENT 7%			RELATIVE DISAGREEMENT 1.7%		ABSOLUTE DISAGREEMENT 16.4%		ANTI-COMPLEMENTARY 2.5%	
Wassermann	-	±	-	±	-	-	±	-	-	-	Anticomplementary	
Precipitation	-	±	-	±	-	-	±	-	-	-	±	-
	2335	28	5142	685	40	91	84	1594	118	54	6	206
	22.5%	0.3%	49.7%	6.6%	0.4%	0.9%	0.8%	15.3%	1.1%	0.5%	0.06%	2%
	POSITIVE				DOUBTFUL				NEGATIVE			
Wassermann	5300 = 51%				797 = 7.7%				4020 = 38.7%			
Precipitation	7627 = 73.4%				165 = 1.6%				2591 = 25%			

TABLE II

COMPARATIVE SENSITIVITY OF THE KAHN AND AUTHOR'S PRECIPITATION TESTS IN 1328 SYPHILITIC SERA

	ABSOLUTE AGREEMENT 78.8%			RELATIVE AGREEMENT 5.2%		RELATIVE DISAGREEMENT 1.6%		ABSOLUTE DISAGREEMENT 14.3%	
Kahn	-	±	-	-	-	-	±	-	-
Precipitation	-	±	-	-	±	±	-	-	-
	344	2	701	65	4	6	16	177	13
	26.9%		52.7%	4.9%	0.3%	0.4%	1.2%	13.5%	1%
	POSITIVE			DOUBTFUL			NEGATIVE		
Kahn	718 = 54%			83 = 6.2%			527 = 39.79%		
Precipitation	943 = 71%			12 = 1%			373 = 28.1%		

The contrast between the fine granular appearance of the positive Kahn tests, and the coarse, readily visible clumps of the precipitation reaction under discussion was usually striking. The recentrifugation of all tests about which there was the slightest doubt contributed considerably to the clarity of the results and to the comparative infrequency of doubtful reports.

As expected corollaries of this greater sensitivity, (1) patients with early syphilis under active treatment regularly continue to give a positive precipitation test for weeks and even months after the Wassermann has become negative, (2) old

cases of syphilis whose Wassermann remains weakly positive, varying between positive, negative, and doubtful from week to week, usually give a persistently positive precipitation test, (3) many cases of syphilis, placed upon probation after one or two years' treatment, then Wassermann having become and remained negative, are found to retain small quantities of reagin in their serum, the precipitation test being persistently positive

In this series of 10,383 tests, there was absolutely no evidence that either the Wassermann or precipitation test have selective sensitivity for particular clinical conditions. Titration experiments in our hands indicate that if reagin is present in high concentration, both types of test are positive, if present in small amounts, the precipitation test may give a positive reaction when the Wassermann reaction is negative. Of 2612 tests in this group, in which the two tests disagreed, the precipitation test was more strongly positive in 2370. It is nevertheless true that a few patients were encountered in whom the Wassermann reaction was persistently positive the precipitation test persistently negative. This phenomenon could not be attributed to a selective reactivity of the former in particular clinical conditions, for the cases belonged to no one clinical group being a fair random sample of the entire series of cases.

b *Results in a General Hospital and Dispensary Population (16,228 tests)* — The data summarized in Table III again illustrate the greater sensitivity of the precipitation test, as well as its comparative freedom from doubtful results. The total number of positive reactions in this general group of hospital and dispensary patients was 2117 (13 per cent), as compared with 1432 (8.9 per cent) for the Wassermann reaction. The precipitation test detected 503 positive sera in which the Wassermann was negative, the situation was reversed in only 49, while the incidence of doubtful reactions was 0.7 per cent for the precipitation test, and 1.4 per cent for the Wassermann test.

Qualitatively the same results were obtained in comparing the Kahn and precipitation tests in a small group of patients (Table IV), the Kahn test proving more sensitive than the Wassermann, but distinctly less sensitive than the precipitation test.

There were at least 46 patients with clinically active syphilis in the general hospital dispensary group never before treated or examined in this clinic, in whom the first precipitation test was positive, while the first Wassermann was negative.

TABLE III

RESULTS IN A GENERAL HOSPITAL POPULATION 16,228 WASSERMANN AND PRECIPITATION TESTS

	ABSOLUTE AGREEMENT 93.7%			RELATIVE AGREEMENT 1%		RELATIVE DISAGREEMENT 0.9%		ABSOLUTE DISAGREEMENT 3.4%		ANTI-COMPLEMENTARY 1%	
Wassermann	-	±	+	±	+	-	±	-	±	Anticomplementary	
Precipitation	-	±	+	+	±	±	-	+	-	-	± +
	13814	13	1372	161	11	93	58	503	49	70	3 81
	85.1%		8.5%	1%		0.6%	0.3%	3.1%	0.3%	0.4%	0.5%
	POSITIVE					DOUBTFUL			NEGATIVE		
Wassermann	1432 = 8.9%					232 = 1.4%			14410 = 88.8%		
Precipitation	2117 = 13%					120 = 0.7%			13991 = 86.2%		



(24), doubtful (13), or anticomplementary (9) As is seen in Table V, there is again no evidence of selectivity for a particular clinical condition

TABLE IV  
RESULTS IN A GENERAL HOSPITAL AND DISPENSARY POPULATION 1045 KAHN AND PRECIPITATION TESTS

	ABSOLUTE AGREEMENT 95.8%			RELATIVE AGREEMENT 0.9%			RELATIVE DISAGREEMENT 1.3%		ABSOLUTE DISAGREEMENT 2%	
Kahn	-	±	+	±	+	±	-	±	-	-
Eagle	-	±	+	±	±	±	±	-	-	-
	POSITIVE					DOUBTFUL		NEGATIVE		
Kahn	96 = 9.2%					15 = 1.4%		934 = 89.4%		
Eagle	111 = 10.6%					8 = 0.8%		926 = 88.6%		

TABLE V  
46 CASES OF CLINICALLY ACTIVE SYPHILIS GIVING A POSITIVE PRECIPITATION TEST, AND A NEGATIVE, DOUBTFUL OR ANTICOMPLEMENTARY WASSERMANN REACTION

WASSER- MANN REACTION	PRECIPI- TATION TEST	CLINICAL CONDITION							TOTAL
		CON- GENITAL	PRIMARY OR SEC- ONDARY	NEURO- SYPHILIS	AOERTITIS OR AOERTIC INSUFFIC	GUMMA	KERA- TITIS, IRITIS	BONE	
-	+	1	1	9	6	1	4	2	24
anticomp	+	2	1	3	2	1			9
±	+	2	1	3	4	1	2		13

### III Specificity of the Precipitation Test —

There remains to be answered the essential question of specificity Are these numerous discrepancies between the Wassermann and precipitation tests due actually to the greater reactivity of the latter with syphilitic sera only, or, are they due even in part to nonspecific reactions with normal nonsyphilitic sera?

A *Doubtful Reactions*—As reported in this laboratory, doubtful reactions include any test in which, whether because of adventitious particles in the serum (dirt, denatured protein), dirt in the tube, or indefinite aggregation of the lipid particles, no definite evaluation of the test in terms of positive or negative is possible Any report of doubtful is thus not a diagnosis but an admission on the part of the laboratory that a definite report could not be rendered, calling for a careful serologic and clinical check-up If all subsequent tests are negative, the original doubtful report is presumably due to some technical factor If subsequent tests prove positive, or if the patient presents clinical evidence of syphilis, the doubtful reaction indicates that the serum contained minimal quantities of circulating reagin not enough to give clear-cut aggregation yet enough to cause suggestive clumping of the lipid particles One of the points of superiority of the precipitation test under discussion is the comparatively small incidence of dubious results (1/40 in the general hospital 1/65 in the known syphilitic group) as compared with an incidence of 1/70 and 1/13 for the Wassermann reaction In the total

series of tests, one out of every 26 was Wassermann or Kahn doubtful, while only one in every 107 gave a doubtful precipitation test

That such doubtful reports cannot be ignored is shown by the fact that of the 241 doubtful precipitation tests in which histories were available for study, 179 were on patients previously diagnosed as syphilitic and treated, 165 of the tests being on patients under active treatment, that 4 more of the 285 presented definite clinical evidence of syphilis, that 13 more were Wassermann positive either at the time of the test or upon subsequent tests, in other words, that at least 212 of the total group were probably syphilitic (Table VI). The incidence of false doubtful reactions (reactions on patients presenting no clinical evidence of syphilis, and in whom all subsequent tests were negative), in the entire series was less than 1-1700 (0.06 per cent), if the calculation is based on the general hospital group alone, it was 1-600. Nevertheless, it must again be emphasized that a single doubtful re-

TABLE VI  
ANALYSIS OF THE 10,129 POSITIVE AND DOUBTFUL PRECIPITATION REACTIONS IN 26,611 TESTS

	TOTAL NO	NO FOR WHICH HISTORIES WERE AVAILABLE	LATENTS UNDER OBSERVATION OR TREATMENT AS SYPHILITIC	WASSERMANN POSITIVE AT TIME OF PRECIPITATION TEST	CLINICAL EVIDENCE OF SYPHILIS AT TIME OF TEST	WASSERMANN POSITIVE UPON REPEAT'D TEST	DEFINITE HISTORY OF ANTSYPHILITIC TREATMENT	SYPHILIS PROBABLY FROM HISTORY OF CLINICAL FINDINGS	TOTAL PROBABLE CORRECT TESTS	DATA ON HISTORY INADEQUATE	FALSE REACTIONS	CALCULATED INCIDENCE OF FALSE REACTIONS
Positive Precipitation Test	9844	9476	7627	1372	62	92	235	88	9427	70	28	1/900
Doubtful Precipitation Test	285	241	165	11	4	2	14	16	212	29	11	1/1750
Total	10,129	9,717	7,792	1,383	66	94	249	104	9,639	99	39	1/600

port per se is not diagnostic or even presumptive evidence of syphilis unless supported by clinical or further serologic proof

**B Technical False Positive Reactions**—In any large laboratory, no matter what the technic used, it is inevitable that there be a certain number of technical false positive reactions, positive results obtained on presumably nonsyphilitic individuals in whom all subsequent tests are negative. The possible causes for false reactions in a precipitation test are numerous and varied: adventitious particles of dirt or denatured protein in the serum mistakenly interpreted as lipid aggregates, mislabelled specimens, error in the dilution of the antigen and in the amount added, etc. The more delicate the adjustment of the reaction, the more numerous are the technical pitfalls, and the higher the incidence of these errors in technic and interpretation. Conversely, if a given reaction is characterized by a wide margin of safety in the adjustment of the reagents, time of incubation, etc., there are correspondingly fewer technical false positive reactions. Although they may be

readily detected by repeating every positive reaction before instituting treatment, too high an incidence of such reactions seriously impairs the value of any test.

The incidence of these technical false positive reactions in the general hospital, excluding the group of syphilitic sera, was less than 1/400, that is, 0.25 per cent (Table VII). If this incidence is referred to the total series of 26,641 sera, it becomes less than 1/600. Even this is a maximum figure, inasmuch as many of these cases had only one repeat test and may indeed have been found to be seroposi-

TABLE VII

ANALYSIS OF THE TESTS IN THE GENERAL HOSPITAL AND DISPENSARY POPULATION IN WHICH THE FLOCCULATION REACTION WAS MORE STRONGLY POSITIVE THAN THE WASSERMANN

WASSERMANN	FLOCCULATION	TOTAL NO. OF TESTS	PERCENT FOR WHICH HISTORICAL DATA AVAILABLE FOR STUDY	PREVIOUSLY DIAGNOSED AS SYPHILIS AND TREATED	CLINICAL EVIDENCE OF SYPHILIS	WASSER LATER POSITIVE	SYPHILIS PROBABLE FROM HISTORY OR CLINICAL FINDINGS	TOTAL PROBABLY CORRECT	DATA ON HISTORY INADEQUATE	FALSE REACTION	CALCULATED INCIDENCE OF FALSE REACTIONS IN THE SERIES OF 10,928 TESTS
-	+	503	411	174	37	50	69	330	54	27	1/425
±	+	161	117	45	15	29	17	106	11	0	0
anticomp	+	81	47	16	10	13	2	41	5	1	1/800
Total Precipitation positive and Wassermann inconclusive		745	575	235	62	92	88	477	70	28	1/400
-	±	93	63	7	4	1	15	27	27	9	1/700
±	±	13	12	6		1	1	8	2	2	1/6000
anticomp	±	3	1	1				1			
Total Precipitation doubtful and Wassermann inconclusive		109	76	14	4	2	16	36	29	11	1/600
Grand total		854	651	249	66	94	104	513	99	39	1/250

tive on repeated testing, for it is characteristic of weakly positive sera of syphilitic patients to yield inconsistent, varying results upon repeated tests.

We believe this result compares more than favorably with tests currently used. It is interesting to note that the incidence of technical false positive and doubtful Wassermann reactions was 1/900 and 1/500 respectively, for the general group (Table VIII). To all intents and purposes, therefore, the precipitation test is almost as reliable as the Wassermann test used in our laboratory as regards technical false positive and doubtful reactions.

*C. Biological False Positive Reactions*—These figures, however, supply only a partial answer to the question of specificity. What interpretation is to be placed upon a persistently positive precipitation test in the absence of clinical signs of syphilis? Does such a series of tests invariably indicate the presence of syphilis, if not, with what frequency is it obtained in nonsyphilitic individuals? For reasons

TABLE VIII

ANALYSIS OF THE TESTS IN THE GENERAL HOSPITAL AND DISPENSARY IN WHICH THE WASSERMANN WAS MORE STRONGLY POSITIVE THAN THE PRECIPITATION TEST

WASSERMANN	PRECIPITATION	TOTAL NO. OF TESTS	CASES FOR WHICH HISTORIES WERE AVAILABLE FOR STUDY	PREVIOUSLY DIAGNOSED AS SYPHILIS AND TREATED	CLINICAL EVIDENCE OF SYPHILIS	PRECIPITATION TEST LATER POSITIVE	SYPHILIS UNDOUBT	TOTAL PROBABLE CORRECT	DATA ON HISTORY INADEQUATE	FALSE	CALCULATED INCIDENCE OF FALSE RELATION IN THE SERIES
+	+	49	32	10	2	5	2	19	2	11	1 900
+	+	11	9			6	1	7	2	0	
Total Wassermann positive and precipitation inconclusive		60	41	10	2	11	3	26	4	11	1 900
±	±	13	12	6		1	1	8	2	2	1 6000
±	±	58	37	5	2	1	4	12	11	14	1 525
Total Wassermann doubtful and precipitation inconclusive		71	49	11	2	2	5	20	13	16	1 500
Grand Total		131	90	21	4	13	8	46	17	27	1 325

which will appear in the following discussion, no absolute answer to these questions is now possible, not only for this reaction, but for any laboratory test for syphilis, including the Wassermann reaction. Based on three distinct lines of evidence, however, the author believes the former statement to be true *that a repeatedly positive precipitation reaction is as reliable a criterion of syphilitic infection as a repeatedly positive Wassermann reaction and any patient giving persistently positive tests from a reliable laboratory is to be considered syphilitic, even in the face of a negative Wassermann reaction*.

1 In the first place, among 189 applicants for the blood transfusion donor list, there was only one positive result the remainder being entirely negative. That one case was found to have a darkfield positive chance. Only by testing thousands of such subjects, in whom the incidence of syphilis is almost nil, will we arrive at any incontrovertible conclusion as to the absolute specificity of this or any other test for syphilis, including the Wassermann reaction. These 189 cases alone, although suggestive, are too small a series to justify any definite conclusions. We proceed with more indirect and correspondingly less conclusive evidence.

2 Among 946 white patients admitted to the private wards, or observed in a pay diagnostic clinic, patients in whom the incidence of syphilis is lower than in other hospital groups, there were 19 cases with a positive and 7 with a doubtful Wassermann reaction. Of these, 2 and 2 respectively proved to be technical errors, subsequent precipitation test and Wassermann tests being negative. In 4 others (1 positive and 3 doubtful reaction) the test had not been repeated and no deduc-

tion can be drawn from them. There remained for study 17 cases with a persistently positive Wassermann test. The clinical analysis of these cases is given in Table IX. As is there shown, in 4 (23 per cent) of these there was neither history nor clinical evidence of syphilitic infection.

TABLE IX

ANALYSIS OF RESULTS IN 946 WHITE PATIENTS IN THE PRIVATE WARDS OR DISPENSARY, SHOWING THAT A PERSISTENTLY POSITIVE PRECIPITATION TEST, EVEN IN THE FACE OF A NEGATIVE WASSEPMANN REACTION, IS AS RELIABLE A CRITERION OF SYPHILIS AS A POSITIVE WASSEPMANN REACTION

	TOTAL NO	TECHNICAL ERROR (POSITIVE ON ONE OCCASION ONLY)	CLINICAL EVIDENCE OF SYPHILIS	DEFINITE HISTORY OF SYPHILITIC INFECTION AND TREATMENT	SYPHILIS PROBABLE FROM HISTORY OR PHYSICAL FINDINGS	NO CLINICAL EVIDENCE NO HISTORY OF INFECTION OF TREATMENT (LATENT SYPHILIS)
Wassermann positive, precipitation test positive	17	0	8	2	3	4
Wassermann positive, precipitation test negative	2	2	0	0		0
Precipitation test positive, Wassermann negative or doubtful	10	2	2	1	3	2

Accepting the present-day evaluation of a persistently positive Wassermann reaction as pathognomonic of syphilis in a temperate climate, it would thus appear that about one-fourth of all the Wassermann positive cases in this selected series of well-to-do individuals have a *latent* infection, latent even to the point of lacking a history of infection. Every one of these Wassermann-positive cases gave a positive precipitation test. In addition, in the 946 patients there were 8 cases in which the Wassermann was first negative or doubtful, and the precipitation test persistently positive. Two of these presented definite clinical and serologic evidence of syphilis, another was later found to be Wassermann positive, in another case there was a definite history of antisyphilitic intravenous treatment, another later gave a doubtful Wassermann and was clinically and by x-ray findings, probably a case of syphilitic aortitis, another had a hepatitis which cleared under potassium iodide, and had impaired hearing as well, and in only two cases out of 8 was there no evidence or history of syphilis, *approximately the same proportion noted in the Wassermann positive group*.

3. Finally, a clinical analysis of 194 unselected cases in the general hospital and dispensary population in which the precipitation test was persistently positive and the original Wassermann inconclusive, showed that 46 presented clinical evidence of syphilis, 84 others were found to be Wassermann positive upon repeated testing, 20 others gave a definite history of having received antisyphilitic treatment, the majority in our own dispensary, in 7 others syphilis was probable on the basis of the history and clinical findings. In all 157 cases (81 per cent) presented

more or less definite evidence, either in their history, clinical examination, or subsequent Wassermann tests, of syphilitic infection (Table X)

TABLE X

CLINICAL ANALYSIS OF 194 UNSELECTED CASES IN THE GENERAL DISPENSARY AND HOSPITAL IN WHICH THE PRECIPITATION TEST WAS PERSISTENTLY POSITIVE, AND THE INITIAL WASSERMANN INCONCLUSIVE

WASS	PRECIPITATION TEST	TOTAL NO	SYPHILIS PREVIOUSLY DIAGNOSED AND TREATED	CLINICAL EVIDENCE OF SYPHILIS	WASSERMANN SUBSEQUENTLY POSITIVE	SYPHILIS PROBABLE FROM HISTORY OR CLINICAL FINDINGS	NO EVIDENCE OF SYPHILIS (PROBABLY LATENT)
-	+	112	14	24	43	5	26
Anticompl	+	24		9	13		2
±	+	58	6	13	28	2	9
Total		194	20	46	84	7	37 (19%)

These three lines of evidence are considered adequate proof for the contention that a persistently positive precipitation test, even in a patient whose Wassermann reaction is negative, is as definite evidence for the presence of syphilis as a positive Wassermann reaction

## SUMMARY

The flocculation test devised by the author has proved to be considerably more sensitive than either the Wassermann or Kahn reaction detecting 75 per cent of 10,383 known syphilitic sera, as compared with 58.7 and 60.2 per cent for the Wassermann and Kahn reaction. It is comparatively free from doubtful results, only one in 137 tests (0.7 per cent) being dubious, as compared with 1.4 per cent for the Wassermann and the Kahn reactions.

In a general dispensary and hospital population (16,228 tests), there were 2117 positive precipitation tests, as compared with 1432 positive Wassermann reactions. In at least 46 untreated cases of clinically active syphilis, the precipitation test was positive, with the Wassermann negative or doubtful.

The incidence of technical false positive and false doubtful reactions in 16,228 tests in a general hospital and dispensary population was 1/400 and 1/600 respectively. In approximately this proportion of cases some error, either of interpretation or technique, led to a positive or doubtful report on a patient presenting no evidence of syphilis, and in whom all subsequent tests were negative. On the other hand, a persistently positive precipitation test was found to be as reliable an indication of syphilis as a positive Wassermann reaction. In 188 selected individuals presumably nonsyphilitic there was not a single positive reaction, in 946 private patients, there were only 8 with a persistently positive precipitation and a negative or doubtful Wassermann. Six of these presented clinical signs, a definite history of syphilitic infection, or a subsequently positive Wassermann reaction. The proportion (2/8) of patients in this group who gave neither history nor clinical signs of syphilis is almost exactly the same as in the Wassermann positive patients of the same group (4/17). Of 194 patients in the general hospital whose precipitation test was persistently positive, though the Wassermann was at first negative or

doubtful, fully 80 per cent gave clinical evidence of syphilis, had a definite history of antisyphilitic treatment, or were later found to have a positive Wassermann reaction. A persistently positive precipitation test, even in a patient whose Wassermann is negative, is therefore believed to be as reliable a criterion of syphilitic infection as a positive Wassermann reaction. In the opinion of the author, it is in itself sufficient evidence for the diagnosis of syphilis.

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## SOME PROPERTIES OF PNEUMOCHOLIN, A BIOCHEMICAL ANTIGEN\*

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THIS paper deals with a new biochemical substance which has been named Pneumocholin. This name has been derived from the first part of the word pneumococcus and from the chemical substance, cholic acid. Pneumocholin itself is a substance of unknown chemical structure produced by the lysis of pneumococci in one of the bile salts. It is probably a protein cleavage product. Some detailed data on the properties and production of pneumocholin are given in this paper. Pneumocholin has been developed as the culmination of more than two years of work and study in an effort to find some specific antipneumococcus agent. This substance has been found to be nontoxic, easily prepared and possessed of marked antigenic properties, producing a high degree of immunity in animals against infection with the pneumococcus.

### REVIEW OF THE LITERATURE

It was discovered in 1900 by Neufeld<sup>1</sup> that pneumococci were soluble in bile. It was later found that the bile salts, which are conjugate amino acids (amino cholic acids), were the constituents of bile responsible for the pneumolytic properties of bile.

From time to time various workers tried the bile salts in a therapeutic manner against lobar pneumonia and other pneumococcus infections.

Barjot<sup>2</sup> treated four men of the black race who were suffering from pneumonia with a 7 per cent solution of sodium taurocholate, containing 2 per cent of magnesium sulphate, intravenously. He reported his results in 1928.

Another investigator in this field was A. Castellanos y Gonzalez<sup>3</sup> who reported his work in several papers during 1929. This worker treated three children suffering from empyema by injecting bile salts intrapleurally. He also treated a patient having meningitis and one suffering from *Pneumococcus* septicemia. He reported his results as being very good.

Early in 1930 the author treated three cases of typical pneumococcus lobar pneumonia with bile salts intravenously.<sup>4</sup> This work brought out the fact that although the bile salts were effective in rapidly terminating the pneumococcus infection, their local toxic action on the veins at the site of injection was too great.

Work was then directed towards finding a derivative of the bile salts which would have a maximum pneumolytic power and a minimum toxic action on animal tissue. This led to the investigation of sodium dehydrocholate which was the subject of a paper published in June, 1931.<sup>5</sup> It was found that sodium dehydrocholate dissolved pneumococci when they were taken directly from the animal body but failed in dissolving pneumococci that were grown in artificial culture media. Sodium dehydrocholate has not yet been tried in clinical work.

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\*1 received for publication June 29 1932

Early in 1931 work was done with sodium dehydrocholate to determine its effect on pneumococci and on tissues. The results of this work have been reported.<sup>6</sup> It was discovered in this work that pneumococci when treated with sodium dehydrocholate stimulated a degree of immunity when injected into rabbits. These chemically treated pneumococci were found to possess antigenic properties which other types of pneumococcus vaccine do not have. Adequate clinical trial of this particular bile salt derivative, sodium dehydrocholate, in the treatment of pneumococcus infections has not been made.

In 1930, Ross published an important paper.<sup>7</sup> In this he described the use of bile salt dissolved pneumococci to produce a high degree of immunity in rats. He fed the bile salt dissolved pneumococci to the rats in their food. Ross also used macerated and hydrochloric acid treated pneumococci in his work, but these became bile treated when they reached the animals' duodenum. These latter two preparations of pneumococci were also reported to have given some what less effective results than the pneumococci treated with the bile salts initially.

Elton by his work<sup>8</sup> has shown that patients with lobar pneumonia have increased icterus indices and positive Van den Bergh tests. His work shows that bile constituents are increased in the blood of such cases, which supports the theory that bile salts are important in the recovery mechanism.

#### EXPERIMENTAL WORK

The work of this paper deals with the production of pneumocholin, the effect of pneumocholin in the animal body, and other properties of pneumocholin.

#### PRODUCTION OF PNEUMOCHOLIN

Many obstacles had to be overcome in the production of pneumocholin. Large amounts of pneumococci are needed and the pneumococcus does not grow very luxuriantly in artificial media.

The propagation of the pneumococci was effected by growing them on blood agar. The plates were always heavily inoculated.

Pneumococci used in the production of pneumocholin were transferred from the blood agar and grown in a type of hormone broth. This broth was made as follows:

Meat extract	3 gm
Pepton	20 gm
Sodium chloride	5 gm
Distilled water	1000 cc

These ingredients are placed in a flask, then two pieces (about two cubic inches) of fat-free beef heart or veal are added. The flask and ingredients are now placed in the autoclave for fifteen minutes at 15 pounds' pressure. Adding the meat in this manner lessens the amount of precipitate that is formed in the media. After removing the flask from the autoclave the media is adjusted to a  $P_H$  of 8.4 and allowed to stand overnight. The broth is now decanted and poured in smaller flasks or tubes. A chip of marble is placed in each tube or flask and the broth is again autoclaved at 15 pounds' pressure for twenty minutes. The standard hormone broth is not very satisfactory as it usually contains more precipitate, but it grows the organisms well. Ascertic fluid broth and serum broth are excellent for growing the pneumococci.

The broth is now inoculated with pneumococci. This is done by cutting out a square of media with a platinum wire loop from a blood agar plate containing pneumococcus colonies and placing this piece of media in the broth.



Maximum pneumococcus growth is usually obtained in forty-eight hours at 37° C

This pneumococcus broth culture is now filtered through a sterile, coarse paper filter into a sterile flask. This removes any solid particles or precipitate from the broth and allows most of the organisms to pass through. The pneumococcus broth culture is now placed in centrifuge tubes and centrifuged at high speed until the broth becomes clear and the organisms are precipitated in the bottom of the tube. The broth is now poured out of the tubes or drawn out with a vacuum pump. The centrifuge tubes are refilled with the pneumococcus broth culture and the process repeated until enough pneumococci are obtained, packed in the bottom of the tubes. The centrifuge tubes and contents are kept sterile with caps. Paper caps were used in this work.

When the last portion of broth has been removed from the centrifuge tubes, the pneumococci are resuspended in 0.85 per cent sodium chloride solution containing 2 per cent of sodium taurocholate\*. The centrifuge tubes are now placed in the incubator for twenty-four hours. This product is pneumocholin and is ready for intravenous injection. It is well to seal the pneumocholin in ampules for later use.

In the above work 30 c.c. of a solution of sodium taurocholate 2 per cent and sodium chloride 0.85 per cent was added to each 0.1 c.c. of packed organisms.

The bile salt, sodium taurocholate was selected for this work because it is the least toxic of the natural bile salts, is effective in producing lysis and is readily available commercially. Many of the cholic acid salts or conjugates would be available to produce pneumocholin.

Pneumocholin itself has a certain solubility and if made too concentrated will produce a cloudy suspension and gradually precipitate. This usually happened when the above product was placed in the refrigerator. On mild heating, solution would again take place. Further purification of pneumocholin can undoubtedly be effected by taking advantage of its relative insolubility. Further quantitative work and other chemical experiments will have to be performed in order to standardize pneumocholin.

#### ANTIGENIC EFFECT OF PNEUMOCHOLIN IN THE ANIMAL BODY

Due to the amount of work involved, experiments thus far have been confined to Type I pneumococci.

Virulence was maintained by recovering the pneumococci from the control rabbits which died, or by passing the organisms through mice.

Virulent pneumococci as used in the experiments were recovered from an animal dying from a pneumococcus infection. The organisms were passed from the animal to a blood agar plate, to broth. No more artificial growth than this was allowed. The broth was then used to inject the experimental animals. Incubation in every case was for twenty-four hours. In inoculating broth, it was found effective and reliable to cut a small square from the blood agar plate with a platinum wire loop and to transfer this block of media to the flask of

\*The sodium taurocholate used in this work was obtained from the Difco Laboratories Detroit, Mich.

broth If only small amounts of pneumococci are transferred, sometimes growth will not occur For this reason the above method is used

The organisms used in this work were obtained in pure culture \* When the organisms were received they were passed through mice to enhance their virulence The identity of the organism was checked culturally, serologically and tested for bile solubility

Rabbits which died were autopsied in each experiment and the pneumococci recovered by streaking on blood agar plates

Care was taken to have but one variable factor in each experiment

Injections were made intravenously The pneumocholin was made in the manner described above

The rabbits used in the following experiments were of fairly uniform size, ranging from 2 to 3 kg in weight Therefore the factor of weight was ignored

#### EXPERIMENT I (TABLE I)

*Effect of Pneumocholin (Five Minute Experiment)*—This experiment was performed to see if pneumocholin would save rabbits from pneumococci when the two were administered intravenously at about the same time As the experiment shows, the pneumocholin did not protect these rabbits

On the hypothesis that a certain length of time was necessary for the pneumocholin to stimulate the production of immunity the following experiments were undertaken

TABLE I  
EFFECT OF PNEUMOCHOLIN (FIVE MINUTE EXPERIMENT)

RABBIT	FEBRUARY 3, 1932 PNEUMOCHOLIN		PNEUMOCOCCI*	RESULT DAYS
1 C			0 1 cc	D 1
2	0 5 cc	Interval	0 1 cc	D 1
3	0 5 cc	5 Min	0 1 cc	D 1
4	0 5 cc		0 1 cc	D 2

\*Twenty-four-hour broth culture Type I Injections made intravenously  
C = control D = died

#### EXPERIMENT II (TABLE II)

*Effect of Pneumocholin (Seven and Nine Day Experiment)*—It was found that pneumocholin produced an immunity to large fatal doses of pneumococci when they were injected seven and nine days following the pneumocholin The test rabbits in this experiment were not made sick by either the pneumocholin or the fatal doses of organisms The control rabbit died in two days

The pneumocholin used in this experiment was seven days old It was kept in the refrigerator in a cotton stoppered flask

#### EXPERIMENT III (TABLE III)

*Lack of Immunity in One or Two Days*—When Experiment II revealed the fact that pneumocholin produced a marked immunity against pneumococci, it was thought desirable to determine as accurately as possible just when the immunity appeared To this end Experiment III was performed to see if any immunity developed in one or two days This experiment proved that no

\*The pneumococci were obtained from the Mulford Laboratories of Sharp & Dohme Philadelphia Pa

demonstrable immunity was developed in this length of time Experiment IV which follows, is considered the most important experiment in this work It shows very definitely when the immunity induced by pneumocholin appears

TABLE II  
EFFECT OF PNEUMOCHOLIN (SEVEN AND NINE DAY EXPERIMENT)

RABBIT	FEBRUARY 9, 1932 PNEUMOCHOLIN	INTERVAL DAYS	PNEUMOCOCCI*	RESULT DAYS
5	1 cc	7	02 cc	S
6	2 cc	7	02 cc	S
7	1 cc	9	04 cc	S
8	2 cc	9	02 cc	S
9 C			02 cc	D 2

Pneumocholin used was made on February 2 1932

\*Twenty-four-hour broth culture of pneumococci given intravenously  
S= survived C= control D= died

TABLE III  
LACK OF IMMUNITY IN ONE OR TWO DAYS

RABBIT	FEBRUARY 22, 1932 PNEUMOCHOLIN	INTERVAL DAYS	PNEUMOCOCCI*	RESULT DAYS
10	2 cc	1	02 cc	D 1
11	2 cc	1	04 cc	D 1
12	5 cc	1	04 cc	D 1
13	5 cc	2	02 cc	D 1
14	5 cc	2	04 cc	D 3
15	4 cc	2	04 cc	D 3
16 C			02 cc	D 2

\*Twenty-four-hour broth culture pneumococci Type I injected intravenously  
C= control D= died Pneumocholin made on February 2 1932

#### EXPERIMENT IV (TABLE IV)

*Immunity Appears Between Three and Four Days*—This experiment was devised in order to determine when immunity appears following an injection of pneumocholin Injections were made intravenously After giving pneumocholin, intervals of from three to seven days were allowed to elapse, and on each of these successive days a group of rabbits including a control, were given fatal doses of organisms

The experiment showed that immunity began to appear on the third day and on the fourth day was great enough to save all the test animals

The organisms used were very virulent and were given in doses designed to kill the controls in a short time This was necessary to make the results of the experiment clear cut

A series of experiments done at different times have been collected here as Experiment IV In this paper there are nine experiments which are clear cut and show definitely that pneumocholin produces a marked immunity to the pneumococcus These experiments confirm each other in every instance where four days or more elapse after injecting pneumocholin

#### EXPERIMENT V (TABLE V)

*Immunity Present After 49 Days*—This experiment was performed to determine if the immunity to pneumococci, induced by the pneumocholin, lasted for an appreciable length of time The experiment shows conclusively that the immunity which these rabbits acquired in Experiment II was still present forty nine days later Since the immunity was so definite after this

TABLE IV  
IMMUNITY APPEARS BETWEEN THREE AND FOUR DAYS

RABBIT	PNEUMOCOLIN	INTERVAL DAYS	PNEUMOCOCCI*	RESULT DAYS
17	2 cc	3	03 cc	S
18	2 cc	3	03 cc	D 2
19 C			03 cc	D 1
20	2 cc	4	03 cc	S
21	2 cc	4	03 cc	S
22	2 cc	4	03 cc	S
23 C			03 cc	D 1
24	2 cc	5	02 cc	S
25	2 cc.	5	02 cc	S
26 C			02 cc	D 1
27	2 cc	6	1 cc	S
28	2 cc	6	1 cc	S
29	2 cc	6	1 cc	S
30 C			1 cc	D 4
31	2 cc	7	03 cc	S
32	2 cc	7	03 cc	S
33	2 cc	7	03 cc	S
34 C			03 cc	D 2

\*Twenty-four-hour broth culture Type I injected intravenously  
C = control D = died S = survived

TABLE V  
IMMUNITY PRESENT AFTER FORTY NINE DAYS

RABBIT	FEBRUARY 9, 1932 PNEUMOCOLIN	INTERVAL DAYS	MARCH 30, 1932 PNEUMOCOCCI*	RESULT DAYS
5	1 cc	49	1 cc	S
6	2 cc	49	1 cc	S
7	1 cc	49	1 cc	S
8	2 cc	49	1 cc	S
35 C			1 cc	D 2
36 C			1 cc	D 4

\*Twenty-four-hour broth culture Type I injected intravenously  
C = control S = survived D = died

length of time it probably lasts much longer How long it will last will remain for future experiments to demonstrate

#### EXPERIMENT VI (TABLE VI)

*Pneumocholin Tried Against Septicemia*—In this experiment the organisms used were grown on blood agar plates for four times after recovering them from a control rabbit which died, and then grown for twenty four hours in broth Pneumocholin was given intravenously one day following the organisms The control rabbit died in seven days and the test rabbits survived although they became sick These test rabbits did not eat well for several days and lost weight They eventually recovered and regained their weight The test rabbits were saved from a fatal termination which would have been produced by a pneumococcus septicemia It is of course not known yet whether pneumocholin could have any effect on the course of a lobar pneumonia already established

This experiment suggests that pneumocholin might be useful in a therapeutic way because of the short time in which immunity is developed Vaccines of dead or avirulent pneumococci do not produce any practical degree of immunity but it appears that the broken down pneumo

cocci induce immunity in a short time. Although whole pneumococci do not act as effective antigens, bile salt dissolved pneumococci do. It can only be speculated as to what occurs when pneumococci are dissolved in bile salts. Probably protein cleavage products of some kind are formed. It will remain for advancing organic chemistry to fully explain pneumocholin.

TABLE VI  
PNEUMOCHOLIN TRIED AGAINST SEPTICEMIA

RABBIT	FEBRUARY 23, 1932 PNEUMOCOCCI*	FEBRUARY 24, 1932 PNEUMOCHOLIN	RESULT DAYS
37 C	0.4 cc		D 7
38	0.4 cc	2 cc	S
39	0.4 cc	2 cc	S
40	0.4 cc	2 cc	S

\*Twenty-four-hour broth culture Type I attenuated given intravenously. Pneumocholin made on February 2, 1932.  
C = control S = survived D = died

#### EXPERIMENT VII (TABLE VII)

*No Immunity Produced by Avirulent Organisms*—In this experiment, test rabbits were given several intravenous injections of avirulent but living pneumococci at intervals of five to seven days. In the case of two rabbits, Rabbits 41 and 43, four injections of avirulent organisms were given. In the case of Rabbit 42, only two injections of avirulent organisms were given. Seven days after the last injection of avirulent pneumococci the test rabbits and a control were given doses of virulent pneumococci. The results are shown in Table VII. The avirulent pneumococci did not produce any appreciable immunity in the test rabbits.

It appears that whole pneumococci do not have the antigenic properties that bile salt dissolved pneumococci have. This of course is not new and merely confirms previous findings and general clinical experience with pneumococcus vaccines.

A theory advanced here is that the bile salts may dissociate the pneumococcus protein from the pneumococcus carbohydrate and thus allow the free pneumococcus protein to act as an antigen.

TABLE VII  
NO IMMUNITY PRODUCED BY AVIRULENT ORGANISMS

RABBIT	AVIRULENT PNEUMOCOCCI*				PNEUMOCOCCI** MARCH 30, 1932	RESULT DAYS
	MARCH 4, 1932	MARCH 9, 1932	MARCH 16, 1932	MARCH 23, 1932		
41	0.2 cc	0.3 cc	0.4 cc	1 cc	1 cc	D 2
42			0.4 cc	1 cc	1 cc	D 4
43	0.2 cc	0.3 cc	0.4 cc	1 cc	1 cc	D 2
44 C					1 cc	D 2

\*Twenty-four-hour broth culture avirulent strain

\*\*Twenty-four-hour broth culture Type I virulent strain

C = control D = died

#### EXPERIMENT VIII (TABLE VIII)

*Effect of Heated Pneumocholin*—This experiment was performed to see if pneumocholin was heat stable (at 56° C for thirty minutes). The results show that pneumocholin is stable to this mild degree of heat. As this degree of heat kills most vegetative forms or pathogenic organisms, the method is available to safeguard the sterility of pneumocholin. The pneumocholin used in this and the previous experiments was made on February 2, 1932 showing that this substance remains stable for a period of time, over three months so far.

TABLE VIII  
EFFECT OF HEATED PNEUMOCHOLIN

RABBIT	MAY 14, 1932 PNEUMOCHOLIN*	MAY 19, 1932 PNEUMOCOCCI**	RESULT DAYS
5 C		0 3 cc	D 2
34	2 cc	0 3 cc	S
54	2 cc	0 3 cc	S
56	2 cc	0 3 cc	S

\*Pneumocholin was heated at 56° C. for thirty minutes

\*\*Twenty-four-hour broth culture Type I given intravenously

C = control S = survived D = died

#### EXPERIMENT IX

*Intravenous Injections in Man*—Pneumocholin as made above was filtered through a Berkefeld N filter, sealed in sterile ampules and then subjected to heating at 56° C. for thirty minutes

This product doubly protected against contamination was cultured on various media including blood agar and was found to be sterile

Eight cubic centimeters of pneumocholin prepared and checked as above were administered intravenously to each of ten people. There were no harmful effects whatever, either locally or generally

#### DISCUSSION

Whole pneumococci lack adequate antigenic properties. This is demonstrated by an experiment in this paper and in general by the experience of the medical profession with pneumococcus vaccines

The pneumococcus when dissolved with a minimum and negligible amount of bile salt forms a substance having marked antigenic properties. This substance, called pneumocholin, is nontoxic either locally or generally when injected intravenously. Subcutaneous injection is not advised

Although pneumocholin was made in this work with pneumococci and sodium taurocholate, the idea of using sodium dehydrocholate as in former work<sup>6</sup> has not been entirely abandoned. From a certain viewpoint, however, it is probably best to use pneumococci which are completely dissolved when preparing pneumocholin for intravenous injection. The soluble type of pneumocholin as made with sodium taurocholate is also filterable. Filtration can be used as an extra safeguard to insure sterility

Sterility of pneumocholin can be maintained by observing aseptic technique in its preparation and also by pasteurization

It has been found that pneumocholin is stable for a period of months when kept in the ice box in cotton stoppered flasks. Sealed ampules of pneumocholin are being kept for experiments at a later date. Some of these ampules are kept at room temperature and some in the refrigerator

The experiments show that when pneumocholin is injected intravenously, immunity appears between the third and fourth day

Work with Types II, III and Group IV pneumococci will be made the subject of a future paper

As the bile salts dissolve all types of pneumococci it is believed that an effective pneumocholin can be prepared for each type. It is further believed that pneumocholin is not type specific. This however remains to be demonstrated.

There are three potential uses of pneumocholin. The first and most important use would be for prophylaxis. The extent to which pneumocholin might be useful as a prophylactic agent will depend upon the length of time that immunity is conferred. How long the immunity will last can only be determined by future experiments and experience. If immunity were to last only for three or four months it would be worth while to use pneumocholin, especially in such places as military camps and during epidemics. Doctors, nurses, and others exposed to pneumonia patients could well afford to use such a prophylactic agent. If immunity conferred by pneumocholin lasts for a longer time, it will be a correspondingly more valuable agent.

A second possible use would be for therapy. It may be that pneumocholin can be used in the treatment of pneumococcus infections to increase the body's resistance. The experiments indicate that fatal terminations could not be intercepted unless there were a grace period of three to four days, after treatment with pneumocholin. Therapeutic experiments will of course be necessary to prove or disprove this theory.

A third use would be for antiserum production. This, so far, is entirely an hypothetical proposition. It is possible that pneumocholin will be found useful in the production of antisera when injected into animals, in a manner analogous to the production of diphtheria or scarlet fever antisera.

#### CONCLUSIONS

1. Pneumocholin is a purified product, produced by solution of pneumococci in a bile salt.
2. Pneumocholin has remained stable in time, under conditions of refrigeration. The maximum time in which it will be stable has not yet been determined.
3. Pneumocholin has remained stable to moderate heat, 56° C for one-half hour (pasteurization).
4. Pneumocholin, when injected intravenously in rabbits, induces a very effective immunity to pneumococci after three or four days. Only pneumocholin made from Type I pneumococci has been investigated.
5. Pneumocholin promises to be a valuable prophylactic agent against pneumococcus infections and produces no deleterious effects when injected intravenously.

NOTE.—The work of this paper was performed in the laboratory of the Veterans Administration Hospital, Cortesville, Pa. It was done after working hours and at the author's expense.

I wish to thank Dr. Philip B. Matz and Dr. A. H. Pierce for permitting this work to be done in the above hospital and for their words of encouragement.

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## THERAPEUTIC USE OF IODIZED OIL IN PULMONARY DISEASE\*

DEAN B COLE, M D, AND EDGAR C HARPER, M D RICHMOND VA

SINCE the report of Sicard and Forestier,<sup>1</sup> in 1922, relative to the use of iodized oil in the tracheobronchial tree the utilization of this radiopaque substance has become quite general as a diagnostic aid, and current literature is replete with references to its usage. The remedial possibilities from its employment have also been referred to by several authors, and results reported by a few, but the larger percentage of physicians are unaware of the therapeutic value of this agent. During the past five years we have used iodized oil therapy in more than one hundred and fifty patients with pulmonary disease. In 1927 we began giving the oil therapeutic trial in selected patients with bronchitis and bronchiectasis where all other measures had failed. Encouraged by results we broadened its usage until we now unhesitatingly use iodized oil therapeutically in almost any patient suffering with nontuberculous lung disease, and even in selected tuberculous patients. Although Pritchard, Whyte and Gordon,<sup>2</sup> Ochsner,<sup>3</sup> and others had previously reported good therapeutic results, we were not sufficiently impressed with the possibilities of this procedure until convinced, by improvement observed in patients in whom the oil had been used diagnostically.

In our early experience with iodized oil we observed an occasional reaction.<sup>4</sup> Two patients developed symptoms of mild iodism which we attributed to the presence of oil in the stomach.<sup>2</sup> An occasional patient complained of symptoms such as discomfort in the chest and increased cough, probably caused by a deterioration due to age, giving the oil a dark color, possibly with liberation of free iodine. This also was reported by Pritchard, Whyte and Gordon,<sup>2</sup> in April 1926, but was not sufficiently appreciated by us until we encountered similar reactions. Since we have learned to avoid allowing oil to enter the stomach and no longer use any oil that has turned brown we have no such reactions. A reaction similar to that encountered in vaccine therapy with symptoms of aching and chilliness followed by temporary fever is occasionally reported by bronchiectatic patients after the first introduction of oil. We have observed this phenomenon chiefly among patients

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showing some evidence of atelectasis, and have also noted that many of these derive the greatest benefit from the treatment

#### KIND OF OIL USED

Any of the standard iodized oils may be employed, but we now use lipoiodine exclusively, because it is comparatively thin, quite volatile, easy to administer, and free from irritant action. Most patients prefer this oil as it can be taken with but little discomfort. If the oil is heated to 101 to 102° F, it is given more easily and is more soothing to the average patient. Due to the warmth, patients can feel the oil pass through the bronchial tree and many say this is quite soothing. This is especially true of patients having acute laryngitis, tracheitis, asthma, and asthmatic bronchitis.

#### METHOD OF GIVING IODIZED OIL

Various methods for introducing oil into the tracheobronchial tree have been described, but we prefer the supraglottic method as follows.

The patient is seated facing the operator, inclined towards the side into which it is desired to introduce the oil. He is then instructed to relax and breathe quietly, allowing his tongue to rest comfortably on the floor of the mouth. The pharynx, larynx and upper portion of the trachea are then sprayed with a small quantity of 1 per cent cocaine solution and the warm oil (101 to 102° F), is dropped directly into the trachea from a 10 c c syringe with attached laryngeal cannula. By using a laryngeal mirror the oil can be so dropped into the trachea that flooding of the epiglottis and larynx is eliminated, and the possibility of oil being swallowed is avoided. This is possible without the use of a mirror but in our experience, less satisfactory.

#### AMOUNT OF OIL USED

When doubtful as to the patient's tolerance for oil or iodine, and in acute laryngitis and tracheitis, as little as 5 to 10 c c of lipoiodine have been given initially, but usually 10 to 20 c c are administered at the first treatment. Daily progress notes setting forth symptoms and any reaction following each treatment are made. Oil is given daily for two or three or more treatments, or less frequently, depending on symptoms, fluoroscopic appearances and when necessary, x-ray studies. Effort is made to record so far as possible appearances in the tracheobronchial tree, to determine the underlying pathology, the rapidity of absorption or elimination of the oil and the amount of oil remaining or accumulating in the tracheobronchial tree. After the first treatment or course of treatment the patient is advised to return if and when further treatment is desired. Usually within two or three weeks the patient will return for further treatment although occasionally symptoms are relieved for six to twelve months.

#### ELIMINATION OF OIL

We find that the oil is eliminated rapidly in some patients and very slowly in others. In two patients with similar conditions treated alike the oil may disappear in one after a few days and be observed in the bronchial tree of the other after four to six months. It is infrequently expectorated after the first day or two and occasionally a patient reports tasting the oil after periods of weeks or months.

## CONTRAINDICATIONS

Pneumonia, acute lung abscess and acute exudative tuberculosis are believed contraindications for the use of iodized oil, but since we have not tried it in either of these conditions we are unable to speak from experience. Because it is of such proved value in atelectasis of the lung we think it may be valuable in certain pneumonias of atelectatic origin.

Resultant iodism, sepsis, laryngeal edema, drowned lung, and spreading of infection from diseased to healthy lung have frequently been mentioned as dangers<sup>5</sup> but our experience does not confirm this. We believe that with proper care there is but little risk from any of these.

Acute laryngitis, tracheitis, and bronchitis have been mentioned as contraindications, but again our experience does not support this. On the contrary, it is in this group of patients that we believe oil probably has its greatest field of usefulness.

## FUNGUS DISEASE AND VINCENT'S INFECTION

Seven patients with fungus disease of the lung were given iodized oil. Of these there were five with *Moniliasis*, one *Streptothrix* and one *Aspergillosis*. Of these four were markedly improved with complete symptomatic relief and one was apparently cured. In one there was improvement, but not entire relief of symptoms. In one there was no improvement.

Three patients with Vincent's infection were treated with iodized oil, two were cured. One patient with advanced gangrene of the lung improved temporarily after the introduction of oil, but ultimately died following surgical intervention.

	NO CASES	MARKEDLY IMPROVED	IMPROVED	UNIMPROVED	MADE WORSE
Atelectasis	5	5	0	0	0
Asthmatic bronchitis	26	14	12	0	0
Bronchial asthma					
Bronchiectasis	30	20	8	2	0
Chronic bronchitis	44	20	15	9	0
Chronic lung abscess	4	0	4	0	0
Chronic empyema	3	1	2	0	0
Acute respiratory infection (laryngitis, tracheitis, bronchitis)	20	15	5	0	0
Moniliasis	5	5	0	0	0
Streptothrix	1	1	0	0	0
Vincent's Infection	3	2	0	1	0
Aspergillosis	1	1	0	0	0

## CHRONIC LUNG ABSCESS AND EMPYEMA

Iodized oil was used therapeutically in four patients with chronic lung abscess, and three patients with empyema. All were symptomatically improved. Two of the empyemas were tuberculous and were cured by aspiration and irrigation, followed by intrapleural instillation of lipiodol and tincture of gentian violet. Except to state that the oil did none of them the slightest harm we can draw no conclusions from so small a number.

# BRONCHIECTASIS

Thirty patients with severe bronchiectasis were treated with iodized oil. Twenty were markedly benefited, eight slightly to moderately improved, and two showed no improvement. None were made worse.

# CHRONIC BRONCHITIS

Forty-four patients with chronic bronchitis were treated with iodized oil. Thirty-five were improved, nine unimproved.

# ATELECTASIS OF THE LUNG

Five patients with atelectasis were treated with iodized oil, all of whom were very definitely benefited. Usually in a few hours after the introduction of oil, the patients cough and expectorate copiously, and practically all state that they immediately feel better. On the following day some of these patients are symptom free.

# BRONCHIAL ASTHMA

With great caution last year we began using iodized oil therapeutically as a measure of last resort in patients having asthmatic bronchitis with severe recurrent bronchial asthma, where practically all other known remedies had failed or ceased to afford relief. Improvement in some was so prompt and gratifying that we were encouraged to give this treatment further trial and have used it in twenty-six patients. With no patient has there been failure to give some relief. At first we feared the introduction of oil might precipitate an attack of asthma, but in no instance has this happened. Only once has it been necessary to use adrenalin, which is kept constantly available. On the contrary, in many instances the asthma has promptly subsided following introduction of oil. We feel that asthma which is primarily allergic should be treated for this before trying oil, but if and when these measures fail or cease to afford relief, iodized oil should be administered. Many of these patients have emphysema and patchy atelectasis with a complicating lung infection which may be the explanation for the response to iodized oil therapy. We have not used oil long enough or on a sufficient number of asthmatics to know in whom or what type of condition to exclude its use, but commend its trial in all asthmatics not responding to other forms of therapy. It would probably be a safe precaution to do a preliminary skin test for sensitiveness.

# ACUTE LARYNGITIS AND TRACHEITIS

Twenty patients with acute laryngitis and tracheitis have been treated with iodized oil and all experienced relief. In ten the relief of symptoms was immediate, five were relieved within one to two hours, and five other patients experienced some relief, but subsidence of symptoms was slow and gradual. The marked relief of symptoms in these patients has been surprising and gratifying, but the number is too few on which to base any conclusions, however, in no instance has the treatment done the slightest harm.

# ILLUSTRATIVE CASES

*Spirochetosis* —Mr. E. B., an attorney, aged thirty-nine, first consulted us in August, 1930, complaining of chronic cough, expectoration and recent hemoptysis.

Family history was negative. He had had pneumonia during childhood, typhoid fever in 1907, influenza and rheumatism while in France during the war with a recurrence in 1926. A history of morning cough and expectoration dated back for several years. He had noticed that smoking seemed to irritate his throat, causing an inclination to clear the throat with a slight hacking cough, which had become a habit. Hemoptysis two days previously brought the patient for examination. Physical examination gave evidence of an atypical lesion of the left lung with a few small and medium moist râles throughout the lower half. X-ray of chest showed diffuse mottling throughout the left lung which was atypical, but suggestive of an early miliary tuberculosis or a fungoid infection. Repeated sputum studies were negative for tubercle bacilli, but Friedlander's bacillus and numerous spirochetal organisms were cultured from the sputum.

The patient was given massive doses of potassium iodide and small doses of asphenamine and autogenous vaccine, with no improvement at the end of three weeks. We then decided to try iodized oil, introducing 20 c.c. into the tracheo-bronchial tree. Within forty-eight hours, cough and expectoration were relieved and all toxic symptoms disappeared. The patient continued symptom free for four weeks. With return of cough and fatigue he was again treated with the same result. Interval treatments were continued over a period of six months, when he was discharged as apparently cured. He has remained well.

*Chronic Bronchitis*—Miss A. R., an executive, aged fifty-two, first consulted us in June, 1928, because of a spasmodic nonproductive cough occurring and recurring inopportunistically, thus causing her untold embarrassment. Her mother had died of tuberculosis and patient had had pneumonia three times, otherwise family and past history were irrelevant. Present symptoms were of nine months' duration, onset with an acute respiratory infection complicated by empyema of the left antium which had been adequately treated. Physical examination showed small moist râles scattered throughout the bases of both lungs posteriorly, otherwise negative. X-ray of chest showed marked thickening of the bronchi to both bases, otherwise both physical examination and laboratory studies were essentially negative. The patient was given 10 c.c. of iodized oil into the base of each lung. Fluoroscopic observation and x-ray studies showed the oil well distributed in the lung bases. There was evidence of multiple bronchial dilatations, but no bronchiectatic pockets. Patient stated that she received relief from her desire to cough. She was instructed to return whenever she felt the need of more oil, so reappeared in two months stating that she had been relieved completely of her symptoms, but was again becoming annoyed by a desire to cough. She was again given iodized oil and continued to receive these treatments at approximately two month intervals. Meanwhile she remained entirely free from cough, stating that she usually began to feel the need of oil at the end of seven or eight weeks. She remained well and active with no other treatment until the fall of 1930, when she died following an abdominal operation.

*Bronchial Asthma*—Mr. F. O. B., a chemist, aged fifty, first consulted us on Feb. 1, 1932, complaining of asthma. Illness began following influenza in 1918 as spasmodic attacks of cough and expectoration at infrequent intervals. In 1923 he had had a mild left sided pleurisy and in 1928 he had a recurring bronchitis. In

1930 he experienced chest pain that was diagnosed intercostal neuralgia. In 1931 he had an automobile accident with fracture of two ribs followed by the first development of definite asthma. Thorough study was made at an excellent asthma clinic, but no etiologic cause could be found, except pansinusitis and diseased tonsils. The sinuses were adequately treated and tonsillectomy performed but there was no improvement in the asthma. When seen by us he had been unable to eat or sleep and was extremely nervous, due to severe asthma for the preceding ten days. Physical examination showed moderate emphysema with sibilant and sonorous râles throughout the chest. Five cubic centimeters of iodized oil were introduced into each lung. Patient was given ephedrin with a sedative. Two days later he returned somewhat improved but still having asthma. Three days later the patient was admitted to hospital and 10 c c of iodized oil introduced into each lung for three successive days. Asthma was promptly relieved and he has remained comfortable since that time. It has been necessary to administer oil at intervals of five to six weeks. Recently he developed an upper respiratory infection precipitated by exposure and complicated by empyema of the antrum. He was given another iodized oil treatment which relieved the symptoms of laryngitis and tracheitis. Patient has had no further trouble.

*Bronchiectasis*—Miss E. E., a student, aged sixteen, first consulted us in April, 1929, complaining of cough and expectoration. Family history was irrelevant. Present symptoms developed following whooping cough when the patient was three years old. At the age of four years she had developed an empyema of the right antrum which was drained. Following this illness she had repeated attacks of bronchitis and pneumonia which confined her to bed several times each year. During the last four years the patient had had hypothyroidism with an average metabolic reading of -24, requiring nine grams of thyroid extract daily to maintain a metabolic balance. Patient appeared well nourished, but anemic. Examination showed bronchial râles throughout both lungs with showers of small and medium moist râles at both bases. X-ray of chest showed thickening at both bases, suggestive of bronchiectasis. Examination of nose showed heavy mucopurulent discharge into the postnasal space. X-ray of sinuses indicated pansinusitis, which was subsequently adequately treated. Ten cubic centimeters of iodized oil were introduced into the base of each lung, and fluoroscopic and x-ray studies made following its introduction showed numerous bronchiectatic pocketings in both bases. Patient obtained symptomatic relief following the first introduction of oil. Treatments were continued at from three to five week intervals for a period of a year, at which time the chest had shown definite improvement. Patient continued to have recurrent pansinusitis in spite of adequate treatment, so was sent to a less rigorous climate where she remained for almost three years. She then returned to Richmond, and when seen had an acute respiratory infection. Fluoroscopic and x-ray studies showed the tracheobronchial tree entirely free of iodized oil, so treatments were resumed, and more than 100 c c of iodized oil were introduced within ten weeks. She made a most spectacular improvement.

*Acute Respiratory Infection*—Mr. A. V., an executive, aged forty-five, was referred to us for treatment of an acute laryngitis, tracheitis, and bronchitis. He gave a history of what he described as sinus trouble since 1917 since when he has

been treated from time to time without permanent relief. On examination he was found to have an acute laryngotracheobronchitis. He also had an acute upper respiratory infection. X-ray of sinuses showed complete opacity of the left maxillary antrum. Ten cubic centimeters of iodized oil were introduced into the tracheobronchial tree, and patient was referred to an otolaryngologist for treatment of the sinuses, who irrigated the left antrum, removing considerable cystic fluid. The following day the patient reported that he had been completely relieved of chest symptoms following introduction of oil. He required no further treatment.

Mr. O. B., physician, aged sixty-five, consulted us complaining of an acute laryngitis, tracheitis, and bronchitis of three days' duration. He stated that his throat was so irritated that he was unable to rest. He was given 10 c.c. of iodized oil into the tracheobronchial tree. He experienced immediate and complete relief and except for slight expectoration he had no further symptoms. This patient had had no other treatment than the injection of oil.

Mr. L. C. J., aged fifty, was seen in consultation and found to have an acute tracheobronchitis which had not improved after five days of bed rest and medical treatment. He was given 10 c.c. of iodized oil. The following day his physician reported him definitely better and two days later the patient came to our office for another treatment. Following this treatment he was completely relieved.

#### SUMMARY

1 After five years' trial we are convinced that iodized oil has a definite therapeutic value in the treatment of most nontuberculous lung conditions.

2 While the literature contains a few contributions on its therapeutic value in chronic nontuberculous conditions we have been unable after careful search, to find specific reference to its use in acute respiratory lesions. Our experience has led us to believe that even better results are to be obtained in acute infections than in subacute or chronic.

3 It is contraindicated in acute tuberculosis, and in certain acute abscesses of the lung, but has some therapeutic value in lung gangrene.

4 It offers relief in certain patients with intrinsic asthma, afforded by no other drug or form of medication.

5 It is definitely, if not specifically, helpful in the treatment of many fungus lung diseases, where no other form of medication affords relief.

6 The field of iodized oil is broad and merits further study.

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# LABORATORY METHODS

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## THE CORRECTED ERYTHROCYTE SEDIMENTATION TEST\*

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### A METHOD BASED UPON CORRECTION FOR THE RED CELL COUNT

DESPITE the numberless reports concerning the utilization of the erythrocyte sedimentation test which have emanated from all parts of the world since the advent of this serologic test into clinical medicine in 1918,<sup>1</sup> it is surprising how incomparable are the results obtained and how few have been the attempts to free the reaction from certain inherent fallacies. It is surprising that the majority of workers have been content to read the distance which the erythrocyte column falls at the end of one or more hours without regard for certain extraneous factors which may so influence the S R,<sup>†</sup> as to render such a reading quite valueless. The S T would be in more general use were the method standardized, and a definite, corrected figure accepted as the normal.

Even the best methods which have been employed,<sup>2</sup> to <sup>10</sup> do not give results that can be accurately correlated. If the sedimentation test is to gain a permanent place among modern methods of clinical investigation, it must be simple and as free as possible from individual error.

I have been engaged in studies of the S T since March, 1927, and at the outset it was decided to revise the groundwork of the test in an effort to evolve a method which would be simple, inexpensive, and, at the same time free of the many factors of error which are present in the existing methods.

### A CRITICAL STUDY OF EXPERIMENTAL CONDITIONS

Before embarking upon a description of the technic which I have employed, it seems necessary to amplify the foregoing statements by a brief discussion of certain factors which, although liable to cause fallacious S R readings, can readily be avoided.

When we undertake to perform a S T upon any patient we naturally wish to obtain a record of the changes which take place in the plasma of the individual to be investigated. The red cells can be considered as relatively inert bodies in themselves, merely serving as an index of the stability of the suspension which they make when intimately mixed up with the plasma as "blood."

If we approach the problem, keeping this idea well in mind, it is apparent that any extraneous factors must be kept constant as far as possible. When this object has been achieved we obtain a S R reading which is recorded in terms of a figure which depends solely upon factors inherent in the blood to be tested. Only in this

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\*From the Department of Medicine, the Johns Hopkins Medical School.  
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†For the sake of brevity the terms S T and S R will be used throughout to indicate Sedimentation Test and Sedimentation Rate respectively.

way is it possible to perceive the subtle alterations in the plasma which take place when there is a departure from the normal standard of health

The following are the cardinal factors which have to be considered

#### A INFLUENCE OF THE SHAPE OF THE SEDIMENTATION TUBE

Under this heading we include diameter, length, and angle of inclination of the tube. Many types of tubes were experimented with and the S R compared when the width of bore varied, when the height varied and even when the sides were of upright or inverted conical shape. It was found that when the tube is of very fine diameter the upper layer of red cells is ill-defined, due to their uneven fall, and a tube of less than 2 mm internal bore should, therefore not be used. The most constant and even results were obtained in tubes whose length was 6 cm and whose internal diameter was 6 mm. One cubic centimeter of blood in such a tube occupied a column 32.5 mm long. The sides of the tube must be parallel and the lower extremity quite flat.

*Inclination of the Tube*—This is a very important factor, for any deviation of the tube from the perpendicular leads to a marked acceleration of the S R. Lundgren (loc cit) showed experimentally and Eric Ponder<sup>18</sup> demonstrated theoretically that the S R in inclined tubes, is much increased over that in vertical tubes. In fact in a 200 mm column a divergence from the vertical of only  $7.5^\circ$  is sufficient almost to double the S R in a vertical tube.

This point is stressed because in a clinic where the S T was done as a routine, I have seen the blood specimens put aside to sediment in a haphazard way, sometimes vertically but more frequently casually leaning against the side of a cardboard box or in a rack with the tube slanting away from the perpendicular.

#### B INFLUENCE OF THE ANTICOAGULANT

The most widely employed anticoagulant is a solution of sodium citrate in concentrations varying from 2 per cent to 5 per cent. As a general rule it is mixed with blood in the proportion of 1 c.c. of citrate to 4 c.c. of blood. It is fairly obvious that when such a solution as sodium citrate is added to blood, the suspension stability of which is about to be tested, it should be employed in such strength and quantity that it will in itself, exert no appreciable influence upon the erythrocytes. Hence, an isotonic solution should be utilized. This point has only once been emphasized and that was by Westergren. An isotonic solution consists of 0.38 per cent sodium citrate and this should be added in minimal quantity so as to interfere with the S R as little as possible. It was found that 1 c.c. of a 3.8 per cent solution was quite sufficient to prevent 9 c.c. of human blood from clotting (at the same time maintaining isotonicity), whereas most authors have used double that quantity of citrate thereby diluting the blood unnecessarily. Moreover Hessel<sup>17</sup> has shown that the autoagglutinating properties of serum may be very much reduced by the addition of a diluent to the extent of 1:2 and occasionally even at a dilution of 1:4.

In order to determine the relative merits or demerits of the various anticoagulants usually employed, I made many parallel experiments using citrated blood as the standard of comparison in each case. Hirudin or leech extract would seem to be the most perfect anticoagulant, as it obviates the introduction of such compli-



cating factors as dilution and osmotic pressure changes that are inherent in the methods employing citrates or oxalates. However, the use of hirudin is necessarily restricted on account of its high price and in addition a number of my experiments were vitiated by the fact that clotting of the blood specimen commenced before the dried extract had time to act. Heparin also was tried but this, too, is expensive and does not seem to act as efficiently as sodium citrate. A solution of novarsenobillon was tried on several occasions only to be discarded as quite unsuitable.

Potassium oxalate crystals were employed fairly frequently, but owing to the fact that a slight degree of hemolysis occurred on several occasions, it was thought that the shape and surfaces of the red cells must be altered to some extent, thereby diminishing the agglutinability of the erythrocytes. The *S R* is more rapid and the rouleaux formation more extensive in heparinized and hirudinized blood than, in citrated or oxalated specimens.

#### C INFLUENCE OF EXTERNAL TEMPERATURE

The room temperature at which the test is performed must be taken into account. Comparatively little mention of this factor is to be found in the literature until Gordon and Cohn<sup>18</sup> in 1928 and Rourke and Plass<sup>19</sup> in 1929 brought this point into prominence. By making dual experiments with specimens of the same blood, one tube of which has been kept for one hour in an incubator at 37° C and another tube at room temperature (19° to 23° C) during the same period, I have noticed that a much greater speed of fall was recorded at the higher temperature.

The optimum room temperature for the *S T* seems to be 19° to 23° C. Above or below this temperature, marked deviations of *S R* are found.

#### D INFLUENCE OF THE RED CELL CONTENT

This factor has been largely neglected, and it is only during the past year or so that efforts have been made to correct for fluctuations in the erythrocyte count. Fahraeus<sup>2</sup> stated that the *S R* is faster in higher dilutions of blood, and a few others have confirmed this, notably Bonniger and Hermann<sup>20</sup> and Hubbard and Geiger.<sup>21</sup> Cooper<sup>11</sup> says that anemia does not affect the *S R*, and Rubin<sup>22</sup> states that between the limits of 4,000,000 and 5,000,000 erythrocytes per c mm the red cell factor is of no appreciable importance. However, in 1929, Gram<sup>23</sup> published an article, wherein he pointed out the influence of the red cell content upon the *S R*, and in this paper he described a method for correcting the *S R* by fixing a certain hemoglobin percentage (100 per cent) as the normal. On reference to a chart which he had constructed he was able to correct the *S R* on a basis of the hemoglobin content of the blood. Rourke and Einstene<sup>19</sup> in 1930 published an account of a method which aims at correcting the *S R* upon a basis of cell volume. In a report describing the effect of anaphylactoid shock upon the *S R* in animals, Joltram and I<sup>6</sup> in 1929 emphasized the importance of correlating the *S R* figures with the erythrocyte count.

When my work was still in the experimental stage it became obvious that the red cell count played an important part in determining the rate of fall of the sedimenting red cell column. For this reason during the investigation of the first 300 patients five tubes were always set up to sediment containing descending concentrations of erythrocytes from 5,000,000 to 1,000,000 cells per c mm. By this means

a very careful and thorough comparison could be made between the sedimentation rates of the different specimens to be tested throughout a wide range of corpuscular concentration

An easy method of calculating the necessary dilutions involved was obtained by modifying a simple formula given by Blacklock<sup>24</sup> His formula indicates the amount of diluting fluid which it is necessary to add to one volume of fluid of percentage "x" in order to reduce that volume to the desired percentage "y"

It is as follows  $\frac{x}{y} - 1$  where x represents the original percentage and y the desired percentage

This is readily applicable to blood by substituting the number of millions of red cells for a percentage Thus, given 1 c c of citrated blood containing 5,400,000 red cells per c mm, in order to dilute this so that the erythrocyte content becomes 5,000,000 per c mm it is necessary to add  $\frac{54}{50} - 1$  c c = 0.08 c c of plasma As a matter of convenience 10 c c of citrated blood was used and after a red cell enumeration had been done, 5 c c of this blood was transferred to a clean glass container, the other 5 c c was centrifugalized, and the supernatant clear plasma pipetted off to be used as a diluent

If 1 c c of blood, whose content is 5,400,000 red cells per c mm, needs 0.08 c c of plasma, 5 c c will need 0.4 c c Therefore in such a case we add 0.4 c c of plasma to the 5 c c of blood, and there results 5.4 c c of blood whose erythrocyte content becomes 5,000,000 cells per c mm In other words, whatever the blood count may be, the million figure is noted and a similar quantity of blood in cubic centimeters is placed in the containing tube To this is added a quantity of plasma, which is represented in fractions of a cubic centimeter, just as the red cell count figure below the million cipher is represented in fractions of a million

#### Examples —

1 Initial blood count 4,800,000 red cells per c mm 4 c c of blood is placed in the containing tube and to it is added 0.8 c c of plasma The tube now holds 4.8 c c of blood containing 4,000,000 red cells per c mm

2 Initial blood count 6,280,000 red cells per c mm 6 c c of blood is placed in the containing vessel and 0.28 c c of plasma added to it The vessel now holds 6.28 c c of blood whose count will be 6,000,000 red cells per c mm

The next step in the procedure is to mix this red cell suspension intimately by gentle inversion of the tube and, to distribute it among several sedimentation tubes so that by the addition of plasma varying concentrations may be obtained Thus, to obtain 1 c c of blood which will contain 4,000,000 red cells per c mm from a suspension containing 5,000,000, it is necessary to place 0.8 c c of blood from the large tube into the sedimentation tube, and add to it 0.2 c c of plasma

This dilution method works on the principle that if we have 5 c c of a suspension of red cells at 5,000,000 per c mm, and we wish to make from it 5 c c of a suspension containing 4,000,000 red cells per c mm we must take 4 c c of the blood and add 1 c c of plasma Similarly to make a 3,000,000 suspension we take 3 c c of the blood and add 2 c c of plasma

If the original blood count is below 5,000,000 we can concentrate from any given red cell count by employing the same principles for the withdrawal of plasma

*Example*—If the erythrocyte count of the citrated sample is 4,500,000, then by addition of plasma to the red cell suspension in the proportion of 0.5 c.c. of plasma to 4.0 c.c. of blood we obtain a quantity of blood whose concentration is now 4,000,000 red cells per c.mm. To set up a 5,000,000 tube from this suspension one cubic centimeter is placed into each of two small tubes which are then centrifuged slowly for a few minutes so as to separate out a small quantity of supernatant plasma. From each of these two tubes, 0.2 c.c. of the clear plasma is removed by means of a small pipette thus leaving, in each tube, 0.8 c.c. of blood whose concentration is now 5,000,000 red cells per c.mm. After removing one specimen, 0.2 c.c. of the red cell suspension is transferred from it to the other tube, and in this way we have a tube which contains 1 c.c. of blood whose concentration is 5,000,000 red cells per c.mm.

I have proved that this method is satisfactory by constantly checking and re-checking the red cell count at different stages in the process.

It was evident, from the first, that even small variations in the red cell content made a great difference in the S.R., for when a series of tubes was set up containing blood in descending concentration from 5,000,000 to 1,000,000 red cells per c.mm., the S.R. accelerated progressively in proportion to the increasing dilution.

These results were later confirmed, when in 1928, Hubbard and Geiger<sup>21</sup> described the effect upon the S.R. of blood by rough dilution and concentration of the specimen. They point out the difficulties of diluting or concentrating the blood by an accurate method so that a given number of red cells would be present. In their own words "It would not be possible to determine the S.R. and blood count \* \* \* on a given patient and calculate what the rate would be for a different blood count."

A few examples will be quoted of cases taken at random from the list to indicate the influence exerted by the red cell content upon the S.R., and in the appended table there are included 50 cases which are representative of the total series investigated by the concentration and dilution method outlined.

CASE 1—NORMAL RED CELL COUNT 4,680,000

TUBE NO	CONTENTS	S. R. IN 1 HOUR
5	5,000,000 red cells per c.mm.	1.0 mm
4	4,000,000 red cells per c.mm.	6.0 mm
3	3,000,000 red cells per c.mm.	10.5 mm
2	2,000,000 red cells per c.mm.	20.0 mm
1	1,000,000 red cells per c.mm.	27.5 mm

CASE 18—CARCINOMA OF CERVIX UTERI RED CELL COUNT 4,270,000

TUBE NO	CONTENTS	S. R. IN 1 HOUR
5	5,000,000 red cells per c.mm.	9.0 mm
4	4,000,000 red cells per c.mm.	13.5 mm.
3	3,000,000 red cells per c.mm.	20.0 mm
2	2,000,000 red cells per c.mm.	23.5 mm
1	1,000,000 red cells per c.mm.	27.5 mm

CASE 109—ACTINOMYCOSIS OF CECUM RED CELL COUNT 4,190,000

TUBE NO	CONTENTS	S. R. IN 1 HOUR
5	5,000,000 red cells per c.mm.	16.0 mm
4	4,000,000 red cells per c.mm.	19.0 mm
3	3,000,000 red cells per c.mm.	22.0 mm
2	2,000,000 red cells per c.mm.	25.0 mm
1	1,000,000 red cells per c.mm.	27.5 mm

Fig 1 depicts better than any written description the effect of similarly treating blood taken from a normal individual

From these examples it is readily seen what a notable influence the number of red cells has upon the S R in any particular case. It is just in the higher concentrations that a slight variation in the erythrocyte count has an important effect. On many occasions it has been remarked that in a patient who was found to be normal clinically, a rather rapid S R was obtained in the unconnected specimen. The red cell count, however, was in the region of 4,000,000 and when the S R was measured in a tube which contained 1 c.c. of this same blood concentrated up to a 5,000,000 basis, the rate was so much retarded, thereby as to fall within normal limits.

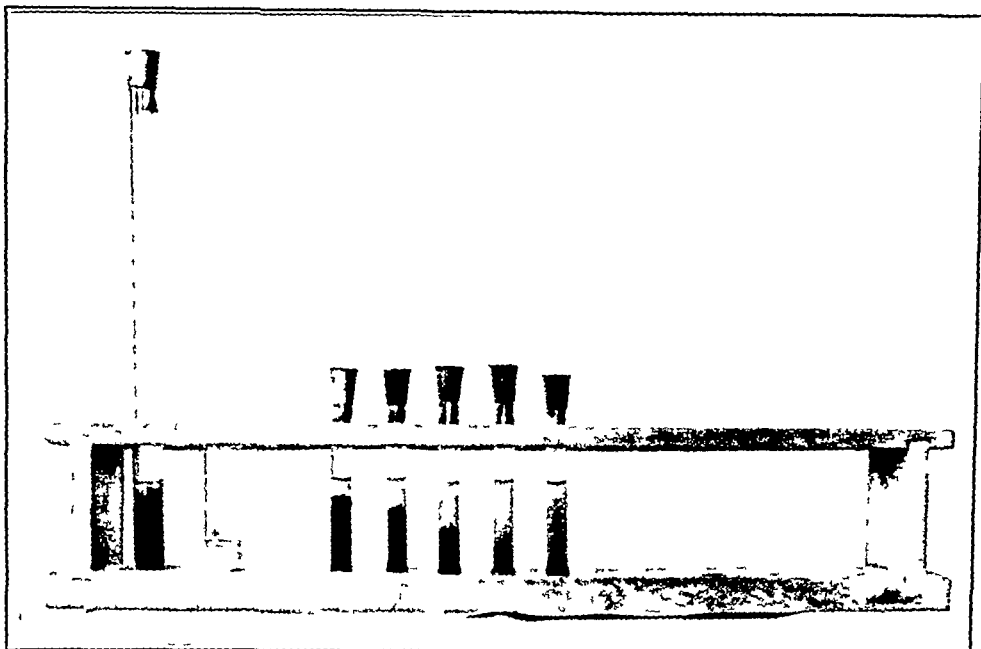


Fig 1—From left to right the tubes depicted contain (1) Reserve supply of blood whose concentration is 5 000 000 red cells per c mm (2) Plasma for dilution (3) S tube containing 1 c.c. of blood whose red cell content is 5 000 000 per c mm (4) S tube containing 1 c.c. of blood whose red cell content is 4 000 000 per c mm (5) S tube containing 1 c.c. of blood whose red cell content is 3 000 000 per c mm (6) S tube containing 1 c.c. of blood whose red cell content is 2 000 000 per c mm (7) S tube containing 1 c.c. of blood whose red cell content is 1 000 000 per c mm

In the tubes of higher dilution the fall is much faster, a S R being obtained which is not quite proportionate to the extent of the dilution, but rather in excess of this ratio. In fact, it was almost constantly found that whether the blood was taken from a normal or a diseased patient the S R in the 1,000 000 tube was 27.5 mm. Moreover in the 3,000 000 and 2 000,000 tubes the difference between the S R in normal and abnormal patients was so small as compared with the wide margin in the 5 000 000 tube that the latter tube was used throughout as the standard of comparison.

Reference to the protocols displays this fact quite well. Whereas Case 1 had a S R of only 1.0 mm in Tube 5, Case 109, frankly abnormal, showed a S R of 16.0 mm. On the other hand the S R in Tube 1 in both cases was the same, namely,

27.5 mm a striking comparison. To a lesser extent a wider margin of difference is found to exist in Tubes 5 than occurs on comparison with the S rates measured in Tubes 4.

There were further reasons for employing the 5 000 000 cell count as the fixed standard. In 1924 Hesser<sup>17</sup> made a serologic study of the red blood corpuscles, especially in relation to agglutination. The rate of sedimentation depends, as can easily be demonstrated, upon the degree of agglutination of the erythrocytes, whereby masses tend to fall faster than small agglomerates. Hesser showed that this agglutination and rouleaux formation occurs best at a high concentration of red cells. If the proportion of cells to plasma was 1.5 in a normal person the agglutination was moderate, although in a rapidly sedimenting blood it was more marked. If the blood was further diluted, the amount of agglutination diminished until at 1:40, in a normal individual, and at 1:80 in a diseased patient, all agglutination disappeared. Thus, in conditions of anemia, where the proportion of plasma to red cell content is increased, the degree of rouleaux formation is considerably diminished. This feature can be well observed if the process of sedimentation be carefully watched through a magnifying lens. In the tubes of higher concentration of red cells, there is a sharp line of demarcation between the upper level of the falling column of cells and the supernatant plasma. In the 3,000,000 tube and the tubes of blood of lower concentration, the large, heavier masses of cell agglomerates fall quickly, leaving in their wake a reddish haze the upper level of which is indistinct. This renders an accurate reading of the sedimentation height impossible. The red haze consists of slowly falling erythrocytes in clusters, which diminish in size to the upper extremity of the cloud, where cells are falling singly or in twos and threes.

Anemia, therefore, renders the S/T valueless, unless it is corrected, for when a count of 1,000,000 cells per c mm is reached, maximal sedimentation with some packing of the cells has always occurred at the end of one hour and before this interval of time the upper level of erythrocytes is too hazy for an accurate reading to be made.

In short, some degree of anemia is not infrequently encountered in disease processes, and if the effect of the pathologic condition upon the S/R is to be studied, some attempt must be made to differentiate the effect of this primary factor from that of the secondary one. A great deal of space has been devoted to the red cell factor in the S/T, but the excuse for this lies in the importance of such a factor which cannot be, and indeed has not been so far, sufficiently emphasized.

#### PERSONAL TECHNIC

The first essential is that all glass tubes, pipettes, and syringes should be kept scrupulously clean and dry. A small flask of sterile, 3.8 per cent solution of sodium citrate is kept tightly corked so as to prevent evaporation and thereby concentration. A fresh solution is frequently made up so that no deterioration is likely to ensue. The actual sedimentation tubes are 6 cm long, 6 mm in internal diameter, and the bottom of the tube is flat. A mark is cut on the glass at a height of 32.5 mm from the base, this mark indicating a content of 1 cc. Lately I have employed tubes graduated in half millimeters but for some time I was content to use a flat metal scale, recording in 0.5 mm, and by placing this alongside the tube it was a

TABLE I

THE EFFECT UPON THE SR OF DIFFERENT CONCENTRATIONS OF RED CELLS THE SR WAS MEASURED AT THE END OF ONE HOUR IN A COLUMN OF BLOOD 32.5 MILLIMETERS HIGH

CASE NO	SEX	DIAGNOSIS	RED CELL COUNT	SEDIMENTATION RATE IN TUBES OF DESCENDING RED CELL CONCENTRATION FROM 6 1 MILLION PER C MM					
				0 MILL	5 MILL	4 MILL	3 MILL	2 MILL	1 MILL
5	M	Normal	4,460,000		0	45	100	180	260
32	M	Normal	4,760,000		10	75	135	195	270
1	M	Normal	4,680,000		10	60	105	200	275
2	M	Normal	4,240,000		10	75	145	210	275
16	M	Normal	4,750,000		15	80	140	200	270
44	M	Normal	4,650,000		20	70	130	200	275
100	M	Normal	4,900,000	10	30	80	145	205	270
201	M	Normal	5,000,000		50	95	155	225	275
93	F	Normal recovery from attack of pelvic cellulitis	4,000,000		0	25	70	125	250
17	F	Normal	4,940,000		15	75	140	210	275
31	F	Normal	4,070,000		25	80	155	215	275
38	F	Normal	4,550,000		30	70	130	210	275
51	F	Normal	5,180,000		40	95	150	220	275
52	F	Normal	4,850,000	10	45	80	150	210	275
64	F	Normal	5,000,000	0	40	100	155	215	275
62	F	Metrostasis	3,980,000	10	45	90	150	210	275
205	F	Normal	5,000,000		50	110	165	225	275
13	F	Normal pregnancy, 9 weeks	3,730,000		0	50	125	195	275
56	F	Pregnancy, 18 weeks	4,110,000		40	95	145	210	275
68	F	Pregnancy, 12 weeks	4,200,000		85	130	175	235	275
47	F	Ruptured ectopic gestation	3,740,000	50	90	140	185	225	275
28	F	Pregnancy, 38 weeks	3,880,000		100	140	180	225	275
12	F	Pregnancy, 24 weeks	4,780,000		100	145	190	235	275
58	F	Pregnancy, 38 weeks	3,970,000	65	110	155	195	235	275
45	F	Pregnancy, 12 hours post partum	4,710,000		120	160	200	240	275
195	F	Pregnancy with toxemia, 36 weeks	4,880,000	135	160	190	220	250	280
53	F	Chronic pyosalpinx	4,820,000	120	160	190	210	255	280
145	F	Acute pyelitis	5,990,000		90	140	185	230	275
120	M	Chronic osteomyelitis	5,140,000		160	190	270	245	280
131	F	Post operative hemioma	3,140,000	125	155	185	220	250	275
137	F	Typhoid fever	4,440,000		125	160	200	240	275
140	F	Convalescent typhoid fever	4,700,000		75	130	185	235	275
94	M	Tbc pleural effusion	4,470,000	85	135	165	200	240	280
118	F	Tbc pleural effusion	5,330,000		145	180	220	245	280
15	F	Tbc peritonitis	4,140,000		110	150	185	225	275
108	M	Actinomycosis of cecum	4,000,000		125	155	200	250	275
109	M	Actinomycosis of cecum	4,190,000	125	160	190	220	250	275
147	F	Subacute nephritis	3,020,000		135	170	215	240	275
163	M	Acute glomerular nephritis	5,200,000		155	180	215	250	280
86	F	Neurotic retroperitoneal sarcoma	3,360,000		125	160	200	235	275
134	M	Hypernephroma	3,960,000		130	170	210	255	275
50	F	Carcinoma peritonei with ascites	3,660,000	50	95	140	185	230	275
141	F	Carcinoma peritonei with ascites	4,370,000		130	165	200	235	275
116	M	Carcinoma of oesophagus	5,500,000		150	185	215	250	280
99	F	Carcinoma of stomach	5,060,000	135	165	195	220	250	280
127	F	Carcinoma of stomach	2,750,000		160	190	220	245	280
101	M	Carcinoma of pancreas	2,730,000		115	150	195	235	275
110	F	Carcinoma of liver	3,500,000		145	170	210	245	275
19	F	Paget's disease of breast	4,930,000		110	150	185	235	275
21	F	Scirrhus carcinoma of breast	5,110,000		85	135	190	230	275

simple matter to read off the height of clear supernatant plasma. A special rack was designed to hold the tubes during sedimentation. There are three rows of holes in the upper platform, and corresponding slots in the base, so that the tubes fit snugly, and are held absolutely vertical during sedimentation.

At one end of the rack are some larger holes to take the larger reservoir tube of blood, and another tube for the plasma with which the dilutions are made. For the purposes of my investigation I had, for a long time set up a series of five sedimentation tubes containing blood in descending concentration from 5,000,000 to 1,000,000 red cells per c mm, and in such instances 10 c c of blood was necessitated. Later, however, it was found that 4.5 c c of blood would suffice, in order to set up one tube with a content of 5,000,000 cells per c mm.

By the usual method, 4.5 c c of blood is withdrawn from an antecubital vein, taking great care not to produce an unnecessary degree of venous stasis, otherwise in a rapidly sedimenting blood some erythrocyte fall *in vivo* will occur (2 and 25)

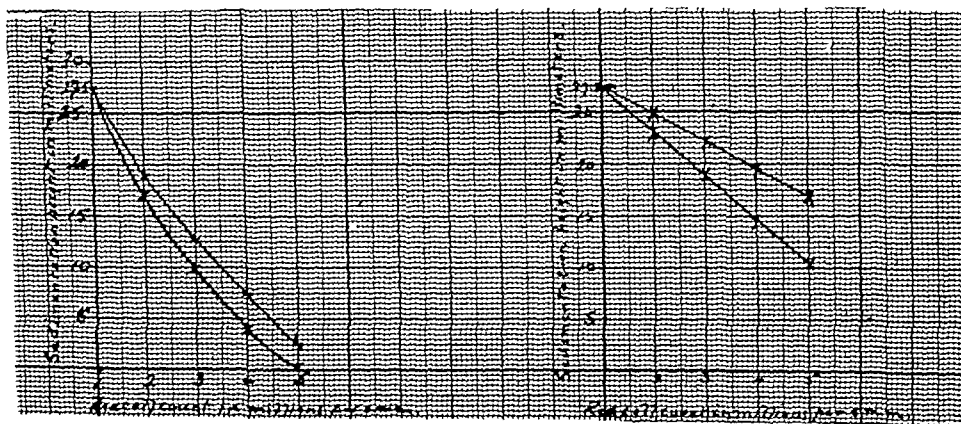


Fig 2

Fig 3

Fig 2 — Illustrates the sedimentation curves obtained from two normal individuals  
 Fig 3 — Illustrates the sedimentation curves obtained from two pathologic cases

This specimen is rapidly introduced into a tube and intimately mixed with 0.5 c c of sodium citrate solution already contained therein. A red cell count on this sample is now made, so that it will be known how to divide, approximately, the 5 c c specimen for dilution or concentration. It is usually expedient to place 2 c c of blood in a small tube, and to centrifuge the other 3 c c in order to obtain plasma with which to dilute the specimen to be tested. The object is to have at least 2 c c of blood, the red cell content of which after appropriate addition of plasma, will register an even million figure, i. e., 6,000,000, 5,000,000, 4,000,000, etc. From this suspension we can, by employing the method just described, readily abstract a sample of blood for a S. T. so that the sedimentation tube will contain 1 c c, the content of which is 5,000,000 red cells per c mm. The sedimentation tube is now corked and an even suspension of blood obtained by inverting and rotating the tube about its long axis. If any air bubbles remain on the surface, they must be burst by the application of a heated glass rod before sedimentation is allowed to proceed. The sedimentation height or height of clear plasma lying above the column of red cells is then measured at the end of one hour.

## A MODIFIED METHOD

In certain cases it is desirable to utilize small quantities of blood for example when frequent repetition of the test is necessary during the observation of chronic pathologic conditions, such as pulmonary tuberculosis or when a S T is performed upon infants and the longitudinal sinus route is chosen. For this purpose a method has been devised, which is simple and rapid so that a large number of sedimentation tubes can be set up at once. This method arose out of the observation referred to that, whatever the S R in a blood specimen at a high concentration might be, a height of fall of 27.5 mm. was almost always attained in the 1,000,000 tube at the end of one hour. During the time when five tubes of descending red cell concentration were set up as a routine, a graphic record was made in many of the cases. Figs 2 and 3 illustrate typical findings in normal (Fig 2) and abnormal individuals (Fig 3).

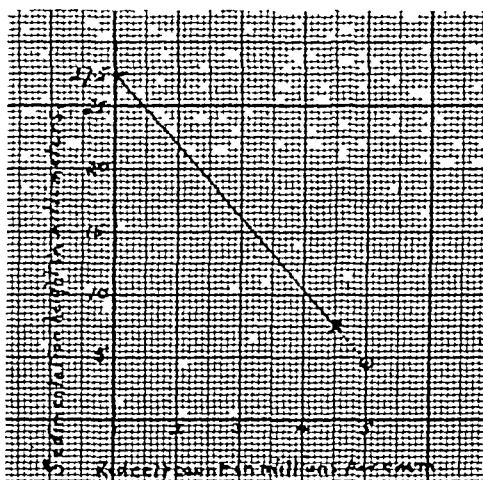


Fig 4—Depicts the procedure for obtaining the corrected S R when the modified technic is employed.

The S R values are plotted upon the graph as ordinates and the red cell counts are abscissae.

In a normal individual, the curve obtained is almost a straight line. When the S R is more rapid the curve described is always a straight line as shown in Fig 3. From a consideration of these curves it is seen that, given a tube of blood at a concentration of 4,500,000 cells per cmm., it is only necessary to measure the S R of this specimen, record the S R figure upon such a graph opposite the red cell count number of the blood, join the point obtained with the 27.5 mm. mark and continue the straight line until it cuts the 5,000,000 ordinate. At the point where this line crosses the 5,000,000 perpendicular, we read off the S R which would obtain if that specimen of blood were concentrated up to a 5,000,000 red cell count.

*Red Cell Count —*

$$\frac{4,500,000 \text{ S R in one hour}}{7.5 \text{ mm}} \quad \text{Corrected S R (to a 5,000,000 basis)} \quad \frac{4.5 \text{ mm}}{5.0 \text{ mm}}$$

*Technic*—A dry 1 cc “Record” syringe is taken and 0.1 cc of 3.8 per cent sodium citrate solution sucked into it. Exactly 0.9 cc of blood is drawn into the



syringe from an arm vein, the citrate and blood are intimately mixed and the contents of the syringe evacuated into an ordinary 1 c c sedimentation tube. A hemocytometer pipette is introduced into the suspension, and enough blood withdrawn for a red cell enumeration, whereupon sedimentation is allowed to proceed in the usual way. If it is necessary to set up several specimens, they may all be placed in the rack to sediment, and the blood counts are performed at any time after the sedimentation height has been read, to suit the operator's convenience.

#### DISCUSSION

For more than five years the method described has been employed by the author during the investigation of a wide range of cases, culled from medical, surgical, gynecologic and obstetric wards. A full account of some of these findings is being reported elsewhere.<sup>27</sup> The number of experiments upon the blood of human beings and animals runs into several thousands, and records to hand amount to upwards of a thousand S tests upon more than 500 patients. This includes a series of 50 cases of *pulmonary tuberculosis*, who were observed over a period of four months during their stay in a tuberculosis sanatorium. Repeated S tests were performed upon these patients, and graphic records were kept of the S R, weight, and temperature. The importance of the corrected S R reading is well seen in such an investigation. Not only does the red cell count differ from patient to patient, but during the course of a chronic disease notable fluctuations in the erythrocyte count are observed, and a false impression of the pathologic condition is obtained if suitable corrections are not made. It was frequently remarked that a sudden rise in the erythrocyte count occurred during a flare-up of the tuberculous process or immediately following collapse therapy. In such instances, and these are but two examples chosen from many others that could be quoted, the true state of affairs is faithfully reflected by the corrected S R figure, whereas the uncorrected figure gives an entirely erroneous picture. When the corrected S R is utilized, the test becomes more accurate and delicate, enabling one to detect changes in the suspension stability of the blood without costly apparatus or extraordinary skill on the part of the observer. Thus the scope of the test is widened and can be utilized in certain specialized ways. In the early diagnosis of tuberculosis, it may be employed as a *specific* diagnostic test, when used in conjunction with the injection of an infinitesimal dose of tuberculin. Another avenue of research is opened in the investigation of hepatic dysfunction by employing the S T and noting the changes in the S R after a protein-containing meal. Without corrected S R figures such special tests as these would not be feasible.

Of the two methods described, the concentration and dilution method, in which one tube of blood is set up containing 5,000,000 red cells per c mm, is necessarily somewhat more accurate than the graphic modification, and this applies especially when the erythrocyte count is in the region of 3,000,000 or less. In the event of such a low cell count, it is urged that the specimen of blood should be concentrated and the S R measured in a tube containing 1 c c of blood at a concentration of 5,000,000 cells per c mm.

The normal one hour S R figures obtained by the methods described are 00 mm to 55 mm with an average of 25 mm in men and 00 mm to 55 mm with an average of 35 mm in women.

## CONCLUSIONS

1 A new procedure is described which endeavors to standardize the erythrocyte sedimentation test and thus eliminate certain fallacies which are inherent in many methods hitherto employed

2 By this technic, which is simple, rapid, and inexpensive, an accurate comparison of the S R may be made in different blood specimens. The S R figure obtained is not influenced by any external factors but is a true reflection of the effect of abnormal states of the plasma upon the suspension stability of a fixed number of erythrocytes

3 The method described necessitates the employment of 4.5 c.c. of blood which is added to 0.5 c.c. of a 3.8 per cent solution of sodium citrate. A red cell count is made of the citrated specimen and then, by means of a simple procedure, the degree of dilution or concentration is calculated which is necessary to bring the sample of 1 c.c. of blood to 5,000,000 red cells per c.mm.

4 The distance that the red cell column falls is measured in millimeters at the end of one hour, and the recorded result is the corrected S R. *Thus in every case investigated, the S R is always spoken of in terms of the distance fallen in an hour by a column of red cells in a tube of 6 mm. diameter containing 1 c.c. of blood at a concentration of 5,000,000 red cells per c.mm.*

5 When it is desirable to take only a small quantity of blood, a modification of this method may be employed which is more rapid and requires merely 1 c.c. of citrated blood. When the S R of this specimen is measured, and a blood count made, the S R figure is corrected to a 5,000,000 red cell basis by means of a graph.

6 This method is not quite so accurate as the previous one, and should not be used when the red cell count lies below 3,000,000. However, it is readily adaptable to the repeated study of any case over a prolonged period of time and especially to the study of children.

7 The utilization of the corrected sedimentation test over a period of more than five years has led me to repose great confidence in the results obtained which have amply justified its adoption.

NOTE: The special sedimentation tubes described in this paper are being made by Arthur H. Thomas Co., Philadelphia.

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## A CLINICAL METHOD FOR SERUM PROTEIN\*

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RECENT researches have called the attention of clinicians to the importance of the determinations of serum protein as a routine clinical measure. Heretofore this determination has been performed largely in research laboratories, and the methods have been difficult and cumbersome for the clinical laboratory technician. There is a demand now that this determination be placed on the basis where it can be carried out by the technician in the average office and hospital laboratory. The following is an attempt to make the present knowledge on the subject available for use in routine clinical work.

The steps are as follows:

- 1 Determine the total nonprotein nitrogen of the serum,
- 2 Determine the total nitrogen of the serum,
- 3 The difference between the two is the protein nitrogen,
- 4 The protein nitrogen multiplied by 6.25 gives the protein.

Nitrogen is most conveniently determined by Kjeldahl digestion and Nesslerization. This method was developed on a micro basis so that small quantities of

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serum may be utilized For the digestion, a micro Kjeldahl flask of 25 c c capacity was made for the purpose by the Fischer Scientific Co of Pittsburgh

*Nonprotein Nitrogen*—Remember to use the serum instead of the customary whole oxalated blood

3 c c of serum in a small flask

21 c c of distilled water

3 c c of 10 per cent sodium tungstate

5 c c  $\frac{2}{3}$  Normal sulphuric acid

Mix and allow the mixture to stand for ten minutes

Filter or centrifuge,

Take 10 c c of the filtrate or supernatant fluid, which is equivalent to 1 c c of serum, place in a micro Kjeldahl flask,

Add a bead or a piece of brick or gravel to prevent bumping,

Add 1 c c of the digestion mixture

Digest according to the Kjeldahl method

When digestion is complete, drop a piece of litmus paper into the flask and then add 10 per cent sodium hydroxide up to the neutral point Be careful not to go past the neutral point, on account of the danger of driving off nitrogen in the form of ammonia with an excess of alkali This neutralization with alkali is necessary because if the mixture is too strongly acid to be nesslerized, it neutralizes all the alkali in the Nessler's solution and fails to bring out the required yellow color

If the liquid is not clear, it must be diluted to a definite volume, say 25 c c, filtered or centrifuged, and an aliquot part, say one-half of the filtrate or supernatant fluid taken, and the factor 2 introduced into the calculation

Nesslerize and read in the colorimeter

In this laboratory where the Klett glass standard colorimeter is used, the calculation is as follows

Multiply the reading by the glass correction, 0.95, this gives  $R$

$15/R \times \text{dilution}$  ( $\times 2$  if clearing of the digested fluid is necessary and aliquot parts taken) gives the milligrams of nitrogen per 100 c c of serum "Dilution" refers to the total number of c c of nesslerized fluid This is a short cut, the full expression is

$15/R \times \text{dilution} \times 100 \times 100$  equals mg per 100 c c serum

Where a Duboseq type of colorimeter is used, with a standard containing 1 mg nitrogen per 100 c c of nesslerized fluid, the calculation is

$S/U \times \text{dilution}$  equals mg of nitrogen per 100 c c serum

*Total Serum Nitrogen*—Place 0.05 c c serum in the micro-Kjeldahl flask This amount may either be measured in a Kahn pipette, in which case it must be delivered below the surface of the distilled water with which it is to be diluted, or 0.5 c c may be diluted to 10 c c with distilled water, and 1 c c of this used for the determination,

3 or 4 c c of distilled water

1 c c of digestion fluid

Digest as in the case of the total nonprotein nitrogen Neutralize as before with 10 per cent sodium hydroxide

Nesslerize and read in the colorimeter

Calculation for Klett colorimeter Reading 0.95 glass correction gives  $R \times 15/R \div \text{dilution} \times 20$  ( $\times 2$  if filtering was necessary) equals mg total nitrogen per 100 c c serum

For a Duboseq type of colorimeter using 1 mg nitrogen per 100 c c of nesslerized standard

$S/U \times \text{dilution} \times 20$  ( $\times 2$  if filtration is needed) equals mg total nitrogen per 100 c c serum

*Digestion Mixture* To 50 c c of 5 per cent copper sulphate solution add 300 c c of 85 per cent (syrupy) phosphoric acid and mix. Add 100 c c of concentrated sulphuric acid and 25 gm potassium sulphate, mix thoroughly

For use, dilute with equal parts of water. Keep well stoppered and protected from light

Digestion flasks after use may be cleaned with strong alkali

*Calculation of Serum Protein*—From the total nitrogen subtract nonprotein nitrogen. The figure gives the number of milligrams of protein nitrogen per 100 c c of serum. Multiplying this by 6.25 gives the number of milligrams of protein per 100 c c of serum. As serum protein is frequently expressed in percentage, this last figure may be divided by 1000 if the percentage of protein is desired

Acknowledgment is made to Miss Violet Payne, technician, for the large amount of detail work necessary to develop and check the above method

## STUDIES IN THE SEROLOGY OF SYPHILIS\*

### X PRECIPITATION TESTS FOR SYPHILIS WITH SPINAL FLUIDS

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WHEN, following the development of the Kahn test, precipitation tests for syphilis began to be generally adopted, it was naturally expected that they would prove as serviceable in the examination of the spinal fluid as they soon proved to be with serum. It was, however, found by many serologists who attempted parallel tests, that whereas precipitation tests with serum were usually more sensitive than the Wassermann reaction, they were distinctly less sensitive when applied to spinal fluids. This inferiority was particularly striking if the Wassermann technique involved the use of a sensitive antigen, ice box incubation, and, most important, the use of large quantities of spinal fluid, 2 to 10 times the amount of serum used in the ordinary serum Wassermann.

The author recently described a simplified precipitation test for syphilis,<sup>1</sup> which in over 26 000 serum tests was found to be considerably more sensitive than either the Wassermann or Kahn reactions and because of the ease of reading results was considered to be more reliable than other precipitation tests currently used. When an attempt was made to apply this test to spinal fluids it was found

<sup>1</sup>From the Syphilis Division of the Department of Medicine, Johns Hopkins Medical School. Received for publication June 1, 1932.

that the spinal fluid Wassermann was as far superior to the precipitation test as the latter was to the Wassermann in the examination of serum. The results obtained in 614 spinal fluids,\* most of them from patients previously diagnosed and treated as syphilitic, are listed in Tables I and II.

TABLE I  
ANALYSIS OF RESULTS IN 614 SPINAL FLUIDS\*

WASSERMANN REACTION IN									FLUIDS	PRECIPITATION TEST (1 cc)				
cc	10	0.8	0.6	0.4	0.2	0.1	0.05	0.02		+	±			
0	0	0	0	0	0	0	0	0	517 5 3 2 2 2 2 15 4 4 10 8 8 6 6 26	(1)**	0			
±	0	0	0	0	0	0	0	0		0	0			
+	0	0	0	0	0	0	0	0		0	0			
+	+	0	0	0	0	0	0	0		0	0			
+	+	±	0	0	0	0	0	0		0	0			
+	+	+	0	0	0	0	0	0		0	0			
+	±	+	±	0	0	0	0	0		0	1			
+	+	±	+	0	0	0	0	0		15	4	5		
+	+	+	+	±	0	0	0	0		4	0	0		
+	+	+	+	+	0	0	0	0		4	4	0		
+	±	+	+	+	±	0	0	0		10	6	1		
+	+	+	+	+	+	0	0	0		8	6	1		
+	+	+	+	+	+	±	-0			8	7	1		
+	+	+	+	+	+	+	+	0		6	6	0		
+	+	+	+	+	+	+	±			26	25†	0		
+	+	+	+	+	+	+	+	+						
Total									Wassermann Results			Precipitation Results		
									+	±	0	+	±	0
									92	5	517	59	8	547

\*In this as in the following tables of this paper

+ indicates a definite positive reaction with no hemolysis

0 indicates a definite negative reaction with complete hemolysis and

± indicates a doubtful reaction with partial hemolysis

\*\*The negative Wassermann reaction was due to some technical error the reaction being positive upon repeated test

†The negative precipitation test was due to some technical error the reaction being positive upon repeated test

Neither of these fluids is included in the discussion of discrepant Wassermann and precipitation tests

TABLE II  
ANALYSIS OF 96 POSITIVE AND DOUBTFUL SPINAL FLUIDS

NO. OF FLUIDS	PRECIPITATION TEST	WASSERMANN	AVERAGE REAGIN TITER OF THE FLUIDS*
5	0	±	<1
24	0	+	3
8	±	+	4½
59	+	+	20-25

\*Wa—positive in 1 cc = 1 unit Wa—positive in 0.2 cc = 5 units etc

\*Fifteen anticomplementary spinal fluids 11 of which yielded a positive precipitation test are not included in the following discussion

## PROTOCOL 1

*Wassermann*—The Wassermann technic was exactly that described in the following paper of this series,\* save that instead of using 0.1 and 0.05 c.c. as in the serum test, the spinal fluids were tested as outlined in the following table, in quantities of as high as 1 c.c.

Spinal fluid, c.c.	1.0	0.8	0.6	0.4	0.2	0.1	0.05	0.02	0.01	0.005
Complement, 1:12 c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2
Antigen 1:200, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Incubation for 4 hours at 0°–6° C., followed by ½ hour at 37° C.

*Precipitation Test*—Exactly as described in preceding papers of this series,\*† save that 1 c.c. of spinal fluid was used instead of 0.4 c.c., as in the serum test, the quantity of antigen suspension remaining the same (0.04 c.c.). As is stated in the text, it has since been found that an even smaller antigen fluid ratio (0.02 c.c. of antigen suspension + 2 c.c. of fluid) results in a more sensitive reaction.

As is there shown, the new precipitation test detected only 67 of the 98 Wassermann-positive or doubtful fluids. The Kahn test proved even less sensitive. Moreover, the outcome of the precipitation test depended upon the amount of reagin present in the fluid, and could usually be predicted from the "strength" of the positive Wassermann reaction. As outlined in Protocol 1, the Wassermann test employed involves the use of graded quantities of fluid, beginning with 1 c.c. of whole fluid, and decreasing to 0.005 c.c. (1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025, 0.01, 0.005). Whenever the Wassermann was positive with as little as 0.05 c.c. of fluid, the precipitation test (carried out with 1 c.c. fluid) was usually also positive. As the amount of the reagin in the fluid decreased, however, negative precipitation tests began to appear. Thus, in spinal fluids which were Wassermann positive only down to 0.4 c.c., positive precipitation tests were the exception rather than the rule, while fluids containing so little reagin as to be Wassermann positive only with 0.6, 0.8, or 1 c.c. of fluid, almost always gave a negative precipitation test. Roughly, the Wassermann proved to be 2½ to 3 times as sensitive as the precipitation test, requiring only 2/5 to 1/3 as much reagin in order to yield a positive reaction.

This has been the experience of most serologists who have attempted to apply precipitation tests to spinal fluid. Recognizing the difficulty, Kahn developed a new procedure for the examination of spinal fluids, involving the precipitation of the spinal fluid globulin (containing the active reagin) by approximately half saturation with  $(\text{NH}_4)_2\text{SO}_4$ , redissolving the precipitate in a minimum volume of physiologic salt solution and using this concentrated globulin solution in a precipitation test.<sup>3</sup> In this laboratory, however, this procedure has proved entirely unsatisfactory, and for two distinct reasons.

Even with reasonable care in the removal of the supernatant  $(\text{NH}_4)_2\text{SO}_4$  solution after the centrifuging of the globulin precipitate, so much  $(\text{NH}_4)_2\text{SO}_4$  remains in the precipitate that when the antigen suspension is added to its solution, there is a nonspecific aggregation, due to excess electrolyte, which simulates the aggregation due to reagin.

A more serious objection is the fact that the procedure usually yields no globulin precipitate in the very spinal fluids in which the ordinary precipitation test is negative and the Wassermann positive—that is, the very fluids in which the

procedure should find its application. Of the 24 fluids in our series falling into this category all but 5 gave a negative or weakly positive Pandy test and a flat or nearly flat mastic curve, in other words at least 75 per cent of these positive fluids contained so little globulin that no significant precipitate would form upon half saturation with  $(\text{NH}_4)_2\text{SO}_4$ . True full saturation of such fluids does yield a precipitate, consisting almost entirely of the normal spinal fluid protein (25 mg per cent or less), plus any reagin or globulin which may have been present, but such a saturated solution has so high a specific gravity that the protein will not sediment clearly upon centrifuging. Moreover, the salt content of any slight sediment obtained is so high as to preclude the ordinary precipitation test.

Nor can it be said that these fluids in which the Wassermann was weakly positive the precipitation test negative, and the protein content either normal or only slightly increased, represent false positive Wassermann reactions, for three-fourths of these cases presented clinical evidence of central nervous system involvement (Table III).

Several other methods of concentrating spinal fluid reagin were then attempted in this laboratory, such as the precipitation of the globulin with  $\text{CO}_2$ , the addition of normal serum to the spinal fluid before precipitation with 50 per cent  $(\text{NH}_4)_2\text{SO}_4$ , and the centrifuging of the lipid particles from spinal fluid at varying  $P_H$ . None of these proved satisfactory.

A significant increase in the sensitivity of the new precipitation test with spinal fluid was finally attained by using much less antigen in proportion to the spinal fluid than is used in the serum test. The latter test calls for a 1:10 antigen serum ratio, by decreasing this ratio to 1:100 (2 c.c. of spinal fluid + 0.02 c.c. of antigen suspension), a considerable increase in sensitivity was effected, without however, attaining the sensitivity of the Wassermann reaction.

The Wassermann reaction therefore remains the method of choice in the examination of spinal fluids. Until some method has been found of increasing the sensitivity of the precipitation test in fluids, the Wassermann cannot be entirely replaced by precipitation tests, no matter how great their technical simplicity, reliability or sensitivity with serum.

Of perhaps only academic interest is the explanation of this puzzling difference in the reactivity of serum and spinal fluid. Why does a precipitation test give a higher proportion of positive reactions with serum than the Wassermann reaction, but less with spinal fluids?<sup>4 to 5</sup>

The answer is to be found in the well known fact that complement fixation is inhibited by serum, as illustrated in the following simple experiment. If one titrates a known strongly Wassermann-positive serum by both the Wassermann and a precipitation test, using a constant quantity of serially increasing serum dilutions, one usually finds that the Wassermann titer is higher, that is, that complement fixation as such is a more delicate test for reagin than the aggregation reaction. If this same experiment is repeated, diluting the syphilitic serum with normal serum instead of with physiologic salt solution, one observes a striking inhibition of fixation, the apparent titer falling to 1/8 and even 1/16 of its original value. The precipitation reaction, however, is practically unaffected by the serum. Clearly, some substance present in normal serum inhibits fixation, and masks



the reagin. The active inhibiting component has been found in this laboratory to be associated with the albumin fraction of the serum protein. Possibly this forms a protective film around the lipoid-reagin particles and prevents the fixation (adsorption) of complement. Be that as it may, given a serum con-

TABLE III  
ANALYSIS OF 29 FLUIDS WITH A POSITIVE OR DOUBTFUL WASSEPMANN REACTION, AND A  
NEGATIVE PRECIPITATION TEST

WASSERMANN REACTION							PANDY	MASTIC CURVE	TOTAL PROTEIN MG %	CLINICAL EVIDENCE OF NEUROSYPHILIS
1	0.8	0.6	0.4	0.2	0.1					
1	±	0	0	0	0	0	0	0	25	Sluggish reflexes
2	±	0	0	0	0	0	—	232210	50	Argyll Robertson pupils
3	±	0	0	0	0	0	0	0(0)0	25	Paresthesias, peroneal palsy
4	±	0	0	0	0	0	0	0	25	None
5	±	0	0	0	0	0	0	0	25 50	Eighth nerve deafness?
6	—	0	0	0	0	0	0	0	25 50	History not available
7	—	0	0	0	0	0	0	0	25	Pupillary changes
8	±	0	0	0	0	0	0	000000	25	General paresis, incipient
9	+	±	0	0	0	0	0	110000	25	None
10	—	+	0	0	0	0	0	0	25	None
11	+	+	±	0	0	0	0	0	25	General paresis, incipient
12	—	+	—	0	0	0	0	0	25	Seventh nerve palsy
13	+	+	—	0	0	0	0	0	50	History not available
14	±	+	—	±	0	0	0	0	50	None
15	—	—	±	+	0	0	±	111000	25 50	Pupillary changes, paresthesia, ulnar hyperesthesia
16	±	+	—	—	0	0	0	0	25	Mental deterioration
17	±	±	±	—	0	0	0	0	25	Tabes
18	±	—	+	±	0	0	±	221000	50	Taboparesis
19	±	+	—	+	0	0	Not done	Not done	Not done	Seventh and eighth nerve palsy
20	—	±	+	—	0	0	++	111000	50	Eighth nerve deafness, paresthesias
21	—	+	+	+	±	0	0	0	25	Third and seventh nerve palsy, pupillary changes, optic neuritis?
22	±	±	+	+	±	0	0	0	25	Argyll Robertson pupils
23	—	+	+	+	—	0	0	0	25	Tabes
24	±	+	±	±	—	0	±	222000	25	Tabes
25	+	+	±	±	—	±	+	554300	75	None
26	±	+	+	—	+	+	+	444211	50	None
27	±	+	—	+	—	±	0	0	25	Optic atrophy—Argyll Robertson pupils
28	+	—	+	—	—	+	0	022222	50	Tabes
29	+	—	—	—	+	+	+	332100	50	General paresis, incipient

TABLE III A

PROTEIN WITHIN NORMAL LIMITS PANDY AND MASTIC TESTS NEGATIVE	VERY SLIGHT INCREASE IN PROTEIN PANDY AND MASTIC TESTS DOUBTFUL	DEFINITE INCREASE IN SPINAL FLUID GLOBULIN
18	4	6

22 out of 28 fluids with a positive Wassermann reaction and a negative precipitation test yield no significant precipitate upon high dilution with 50 per cent (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>.

taining small amounts of reagin, say 1, 2, 4, or even 8 units, this serum inhibitor may prevent complement fixation entirely, while the precipitation reaction, although less delicate than complement fixation, will, since it is relatively unaffected by the inhibitor, be positive. Nor can one escape the inhibition in the Wassermann reaction by using dilute serum for obviously, if one uses 1:5 serum instead of whole serum in the Wassermann reaction, there is not only less inhibitor, but also less reagin, and the net result is a further loss in sensitivity.

The greater sensitivity of the precipitation test with serum as compared with the Wassermann reaction is thus explained, for only those sera which contain enough reagin to overcome the serum inhibition will give a positive Wassermann reaction. In spinal fluids, however, which normally contain less than 25 mg per cent of protein, that is, an amount less than 1/200 the serum protein concentration, and which even in general paralysis rarely contain more than 400 mg per cent (less than 1/15 the serum protein concentration), this inhibiting substance is not present, as is shown experimentally in Table IV. In the absence of the inhibitor,

TABLE IV  
SHOWING THAT SPINAL FLUID CONTAINS LITTLE OR NO INHIBITING SUBSTANCE FOR  
COMPLEMENT FIXATION

DILUENT	WASSERMANN RESULT OF A SYPHILITIC SERUM DILUTED							
	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
NaCl 0.85 per cent	+	+	+	+	+	+	+	+
Normal inactivated serum	+	+	+	+	±	0	0	0
Normal spinal fluid	+	+	+	-	+	-	+	±

the intrinsically greater delicacy of the fixation phenomenon becomes immediately apparent in the results obtained by the spinal fluid Wassermann reaction in comparison with the precipitation test (Table I).

#### SUMMARY

A sensitive ice box Wassermann reaction is intrinsically several times as delicate as the precipitation tests in current use. Serum, however, contains an inhibiting substance or factor, associated with the albumin fraction, which prevents complement fixation without appreciably affecting precipitation, thus causing the latter to be a considerably more sensitive test for small quantities of serum reagin than the Wassermann reaction.

Spinal fluid apparently does not contain this inhibiting factor, probably because its protein content is normally only 1/300 that of serum. The intrinsically greater delicacy of complement fixation becomes immediately apparent the Wassermann reaction detecting 40 to 50 per cent more positive spinal fluids than the precipitation test, despite the fact that the latter detects 20 to 30 per cent more syphilitic sera.

The Kahn procedure of attempting to increase the sensitivity of the precipita-

tion tests with spinal fluid by concentrating the globulin with  $(\text{NH}_4)_2\text{SO}_4$ , was found to be quite unsatisfactory, for reasons described in the text. Some improvement in the sensitivity of the author's spinal fluid precipitation test was achieved by decreasing the antigen-spinal fluid ratio to 1:200, as compared with 1:10 for serum. Nevertheless, the Wassermann reaction remains the method of choice in the examination of spinal fluids, so long as this holds true, it cannot be entirely supplanted by any precipitation test, no matter how great the superiority of the latter with serum.

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## NEW DETAILS OF TECHNIC IN AIR ANALYSIS\*

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THE indirect heat measurement and the respiratory quotient, derived from the analysis of air, have come into such common use that any factors affecting the accuracy of these measurements are of interest. The observations recorded below are a result of several years of work with an apparatus designed and described by Carpenter<sup>1</sup> and also with a similar one as modified by Du Vigneaud.<sup>2</sup> The latter apparatus, described by Du Vigneaud,<sup>2</sup> is adapted for the analysis of expired air as well as outdoor air. A small bulb on the burette separates the range of  $\text{CO}_2$  percentages of outdoor air from those of expired air. The mercury trap was found to be very practical in keeping the burette clean. The mercury and the KOH leveling bulbs were fitted with rack and pinion adjustments and a new potassium pyro-gallate bulb of about 600 c.c. capacity replaced the original one.

The purpose of this paper is not to repeat the excellent directions and precautions already stated by Carpenter,<sup>1, 2, 6</sup> but rather to point out the application and importance of new details heretofore unpublished. I am indebted to Dr. T. M. Carpenter for frequent helpful discussion. The results set forth in this paper are limited strictly to new considerations and to points on which, over a period of

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years, consistent results require a viewpoint and interpretation somewhat different from those of Carpenter. The analyst must of course be his own judge as to the correctness and importance of these considerations.

#### SIMULTANEOUS ABSORPTION OF OXYGEN AND CARBON DIOXIDE

Since the accuracy of the respiratory quotient value depends in part on the magnitude of the percentages of  $\text{CO}_2$  produced and oxygen consumed, there is need for greater accuracy in analysis when respiration chambers are used than when undiluted expired air is analyzed. In the usual method of analysis, an error in the  $\text{CO}_2$  determination causes an error in the corresponding oxygen determination, though if the initial and final volumes are correct, these errors tend to compensate each other as they affect the respiratory quotient. The following "Total absorption" method ( $\text{CO}_2 + \text{O}_2$ ) is short and accurate, and is particularly adaptable to samples taken from respiration chambers, in which the percentage of  $\text{CO}_2$  is relatively low or under any conditions which involve an extensive number of samples.

The usual method followed in an analysis is time consuming chiefly because the air in the capillary tube above the KOH must be freed of oxygen by a process of dilution with nitrogen. In the routine pertaining to oxygen absorption as outlined by Carpenter<sup>1</sup> for instance half of the number of trips of the sample into the pyrogallol are required for this reason and not because of any lack of efficiency in the absorption of oxygen by the pyro.

The modification in method consists of putting the sample directly into the pyro, which of course absorbs both the oxygen and  $\text{CO}_2$ . Ten trips of the sample into the pyro which require about seven minutes, result in complete absorption of these gases. The levels are set as usual with the sample open to the KOH.

Before again putting the sample into the pyro (5 times) to check the completeness of absorption the sample is put once into the KOH to sweep out any oxygen which might have diffused beyond the stopcock above the KOH while the initial volume was being recorded. Concordant readings, taken after 10 trips and after 15 trips as described testify however that no such extensive diffusion takes place during the time required for careful adjustment of the KOH levels when measuring the original sample.

This method of obtaining the total absorption ( $\text{CO}_2 + \text{O}_2$ ) is very short and direct, and minimizes any effects of changes in temperature or barometer during the course of an analysis. Excellent results have been obtained on many scores of samples by this method.

The total absorption value may be obtained independently of any values subsequently obtained for  $\text{CO}_2$ . Thus, the average value of three total absorptions, combined with the average of three  $\text{CO}_2$  determinations gives better data for the determination of oxygen, and a respiratory quotient than do three analyses done in the usual manner. The fact that twice as large a volume of sample is required by the method of determining the  $\text{CO}_2$  and total absorption separately does not detract from its usefulness since the amount of air available for analysis is not ordinarily limited.

If the  $\text{CO}_2$  is measured gravimetrically, only the total absorption value need be determined. Though the apparatus following the determination of total absorption, may be used at once for  $\text{CO}_2$  determinations it must be freed of oxygen

before the order is reversed. The use of one apparatus for  $\text{CO}_2$  determinations and another for total absorption makes this unnecessary.

#### THE EFFICIENCY AND CAPACITY OF PYRO SOLUTION AS AN OXYGEN ABSORBENT

In the preparation of a pyrogallate solution it is obvious that contact with the air should be avoided. This simple precaution has formerly been apparently overlooked. Carpenter states<sup>1</sup> that one filling of the apparatus is sufficient for only 10 analyses but mentions the use of "a long stem glass funnel" in introducing the pyro into the apparatus. The rapidity with which a pyro solution absorbs oxygen has been observed in the course of many routine analyses. After the sample has been forced into the pyro 3 times, more than 99 per cent of the oxygen is already absorbed. This fact, quickly demonstrable with any apparatus, shows the pyro to be an extremely efficient absorbent, especially since the whole sample is never exposed to the pyro at any one time. This rapidity of absorption has not previously been properly evaluated. A proper consideration of the speed and completeness of absorption of oxygen by the pyro when used in a small<sup>1</sup> or large<sup>2</sup> bulb makes it clear that any modification of the form of the bulb or any circulation of the pyro<sup>3</sup> can accomplish but little. Satisfactory mixing of the pyro solution obtains as a result of passing the sample into and out of it. This is indicated by the continued efficiency of the pyro solution. It is also shown by the rapidity and completeness of thorough mixing when two solutions of different colors are placed in opposite sides of the potassium pyrogallate pipette and an air sample forced over in the usual manner. There is therefore in the absence of a circulating device no likelihood that "fresh unused solution"<sup>4</sup> is a factor of any importance. The modified form of the potassium pyrogallate pipette<sup>5</sup> has the alleged purpose of furnishing fresh pyro to the air sample, but no claim is made that any fewer trips of the sample into the pyro are required. Therefore, no time is saved. The larger bulb in the modified form<sup>6</sup> is useful because "75 analyses may be carried out without renewal of the pyrogallate solution." By actual count the writer performed 483 analyses with a similar amount of pyro, checking the analyses from time to time with samples of outdoor air. The pyro seemed as efficient during the last analysis as during the first. The pyro solution was prepared and introduced into the apparatus in the manner described below. Seventy-five or more analyses (instead of ten) may be made from one filling of the small bulb of the earlier form of the apparatus.<sup>7</sup> This is true only if care is taken that the pyro is not largely exhausted by exposure to room air before being actually used for air analysis. One filling of the apparatus may be insufficient even for 10 analyses if the pyro is poured from one container to another in the open air.

In preparing a pyrogallate solution the practice in this laboratory has been to add a weighed amount of pyrogalllic acid to a known amount of strong KOH solution<sup>8</sup> previously placed in an eight-liter bottle and immediately to stopper the mouth with a 2 holed stopper fitted with 2 glass tubes which are closed by rubber connections and clamps. (A little mineral oil may be placed on the surface if care be taken that no oil later is allowed to reach the inside of the air analysis apparatus.)

<sup>1</sup>In the ratio of 10 grams of pyrogalllic acid dissolved in 100 cc. of KOH solution having a specific gravity of 1.2 at 20° C.

To avoid contact of the pyro solution with oxygen in the process of filling the absorption pipette, connect one of the glass tubes in the stopper of the supply bottle with the bottom of the pipette, and the other with a small bottle of pyro. Then elevate the supply bottle so that the pyro runs through one tube into the pipette, while air is drawn into the supply bottle through the other tube and the small bottle of pyro.

As a test of the efficiency and capacity of pyro to absorb oxygen, dry  $\text{CO}_2$ -free air was drawn through known quantities of fresh pyro solution placed in two absorption bottles connected in series. The rate of air flow was about 225 c.c. per minute. The bottles of pyro and the sulphuric acid which followed each were weighed from time to time until no further increase in weight occurred. Three tests showed that fresh pyro can absorb about 15.5 c.c. of oxygen per gram of pyro. Under these conditions the first bottle of pyro let a small amount of oxygen through to the second, even at the beginning, but this amount did not increase at any greater rate until after the first solution had become 65 per cent exhausted.

The conditions of this test were much more severe than those encountered in an analysis, in that the oxygen had only one momentary contact with the pyro. (It may also be pointed out that the maximum quantity of oxygen absorbed in this test undoubtedly exceeds the amount which the pyro will absorb under the conditions of analysis when the last traces must be quantitatively removed.)

Since the efficiency of the pyro is not appreciably diminished until  $\frac{2}{3}$  exhausted, 100 c.c. of fresh solution for an analysis should quantitatively absorb about 1800 c.c. of oxygen. Seventy-five cubic centimeters of pyro in an apparatus having a measuring burette of 40 c.c. capacity,<sup>1</sup> therefore, should be sufficient for at least 150 analyses.

In my practice the number of analyses has never exceeded 80, though it was never demonstrated that the efficiency of the pyro solution was impaired. The inconvenience of introducing a new solution of pyro into the apparatus is considerable. A larger bulb which permits a proportionately larger number of analyses thus serves a very useful purpose.

#### THE ABSORPTION OF WATER BY THE PYROGALLIC ACID SOLUTION

When the sample volume is recorded by reading the height of the mercury in the calibrated burette, the amount of water on the mercury must be subtracted from the apparent volume of the sample. Due to the dehydrating action of the pyro, however, an error is involved if the same amount of water is used in correcting both the initial and final volumes of the sample. The dehydrating effect of the pyro solution has been recognized by others but so far as I am aware no attempt has been reported to evaluate this dehydrating action of the pyro in the computation of the oxygen percentage.

To measure the amount of water removed from the sample by the pyro solution an appropriate volume of sample (nitrogen), about 79 per cent of the total volume of the burette, was forced into the pyro solution 150 times, in exactly the same way as during the determination of total absorption. There was removed by this procedure 0.102 per cent of water as read on the burette, or 0.010 per cent per 15 trips, the number required in the determination of total absorption. The sample was put back and forth into the pyro without any appreciable pause between trips,

thus duplicating the routine ordinarily followed. In addition to this special test the amount of water taken up by the pyro has been verified many times during years of work with this type of apparatus. The length of time that the pyro has been in the apparatus, exposed to the water seal, and the number of oxygen determinations for which it has been used, may possibly affect its dehydrating action, though any such influence has been too small to detect. A small amount of water is used in saturating the initial sample and varies with temperature, but this amount is negligible, especially since no sample was wholly dry when introduced into the apparatus. (The amount of water necessary to saturate a dry sample (40 c.c.) at 23° C. is only about 0.002 per cent as read on the Carpenter burette.)

It is therefore apparent that the volume of water subtracted from the final reading should be 0.010 per cent smaller than that used to correct the original volume. This is an important consideration since this difference is reflected nearly quantitatively in the computed oxygen or total absorption percentage. If the amount of water read, after ample time for complete drainage, is applied to the initial volume of the following sample, the next few water values can be accurately estimated without actually reading the water, checking up the last estimation with the observed amount whenever it is convenient to allow time for complete drainage.

It might be objected that a change in temperature may affect the amount of water condensed. The volume of the sample at the end of an analysis is about 30 c.c. If the room temperature should drop 6° C., say from 23° C. to 17° C., the amount of water condensed would be increased by only 0.00018 c.c. or about 0.0005 per cent as read on the burette.<sup>1</sup>

Any water above the mercury, whether directly on the mercury or clinging to the sides of the burette, occupies volume, and if ignored is measured as gas. In the foregoing test the burette was thoroughly clean, a condition necessary to obtain good analyses, and particularly imperative in this particular test.

The following figures illustrate the methods of computation. The foregoing discussion indicates that the percentage of oxygen (or of total absorption) should be computed as indicated in column B. Column A shows the ordinary method.

TABLE I

	A	B
Initial reading	99.965	99.965
Volume of water	0.186	0.196
Volume of sample	99.779	99.769
Volume after CO absorption	99.935	99.935
Volume of water	0.186	0.196
Volume of sample	99.749	99.739
Volume after O <sub>2</sub> absorption	79.041	79.041
Volume of water	0.186	0.186
Volume of sample	78.855	78.855
Per cent oxygen	20.940	20.932

#### THE ABSORPTION OF OXYGEN AND NITROGEN BY THE KOH SOLUTION

A steady increase in the volume of gas measured (starting with nitrogen) was noted as a result of repeated trips of the sample into the KOH solution. These trips were made in the shortest time practicable, single readings only being taken. Any slight irregularity in the increase in volume is therefore not significant, but

since errors of individual readings are not additive the general situation prevailing is clearly shown (Table II.) The first reading in column B follows the last reading in column A, etc.

After the last traces of  $O_2$  have been removed from the sample, the KOH is then exposed to pure nitrogen on one side and to room air, or about 79 per cent nitrogen, on the other. Many trips of the nitrogen into the KOH under these conditions naturally tend toward equilibrium, or, in other words, toward a loss of nitrogen from the apparatus to the room air, through the KOH. This slight loss is shown by the decrease in the first values listed in columns A, B, C, and D. However, a larger change in the opposite direction takes place in the case of  $O_2$ , due to its greater solubility, and to the conditions of partial pressures which prevail. This increase is shown in the columns of Table II. After the last traces of  $O_2$  have been removed from the sample, the KOH is then exposed to pure nitrogen on one side and to room air, or about 21 per cent  $O_2$ , on the other. Many trips of the sample of nitrogen into the KOH under these conditions naturally tend toward equilibrium, or in other words, toward a gain of  $O_2$  from the room air, as well as a loss of nitrogen to the same. This gain of  $O_2$  is much greater than the loss of nitrogen, as indicated by the data in Table II. Each reading in fact, represents the algebraic sum of the nitrogen lost and the oxygen gained. After the sample has increased 0.06 or 0.07 as a result of 35 trips to the KOH, and is then put into the pyro 10 times to remove the oxygen, the remaining nitrogen is slightly less than before the series of trips was made.

TABLE II

DATA SHOWING THE LOSS AND ABSORPTION OF  $O_2$  AND  $N_2$  FROM A 10 PER CENT KOH SOLUTION

	A	B	C	D
Direct from pyro (10 trips) read at once	79.156	79.135	79.124	79.103
Direct from pyro after one minute	79.159	79.141	79.126	79.105
Direct from pyro after five minutes	79.162	79.143	79.128	
After 1 trip to KOH	79.170	79.148	79.131	
After 2 trips to KOH	79.170	79.148	79.131	
After 5 trips to KOH	79.177	79.159	79.136	
After 10 trips to KOH	79.183	79.173	79.141	
After 15 trips to KOH	79.191	79.173	79.157	
After 20 trips to KOH	79.201	79.186	79.154	
After 25 trips to KOH	79.209	79.189	79.163	
After 30 trips to KOH	79.218	79.195	79.169	
After 35 trips to KOH	79.223	79.200	79.174	

A further indication that the KOH solution absorbs or loses  $O_2$  and  $N_2$ , under the conditions stated above, is that no such change in volume takes place during the regular determinations of  $CO_2$  when the air on both sides of the KOH contains almost exactly the same percentages of  $O_2$  and  $N_2$ .

As a further check on the situation indicated by the above data, nitrogen was put on both sides of the KOH. The compensating bulb was also filled with nitrogen. By opening a screw cock to the room, when the levels were set, this arrangement did not interfere with the sensitivity, or with the compensation.

A routine similar to that followed before showed a temporary increase in volume as the nitrogen was put into the KOH, but this soon ceased altogether. After a few trips into the KOH, and into the pyro to remove oxygen, a series of 50



trips gave no change in volume. The small initial increase is due to oxygen in solution slowly given up to an atmosphere of pure nitrogen.

Leaving the nitrogen in the compensator and on the external side of the KOH, an outdoor air sample was taken in for  $\text{CO}_2$  analysis. Readings were taken after 6, 10, 20, 30, 40, and 50 trips of the sample into the KOH. The readings indicated the following percentages of  $\text{CO}_2$ : 0.047, 0.051, 0.061, 0.068, 0.075, 0.090. This indicates again that the KOH absorbs oxygen under these conditions.

After the nitrogen was removed from the external side of the KOH, and from the compensator, the sample which contained about 21 per cent oxygen was driven into the KOH many times. Then a series of 50 trips resulted in no change in volume.

The practical point in regard to the situation is that since the introduction of nitrogen into the KOH involves a possible error, I believe that the procedure is undesirable and unnecessary. If the sample is taken back slowly from its last trip to the pyro, and a little additional time allowed to complete the saturation (as when taking the initial volume) the error can be minimized.

Since the KOH solution at the end of an analysis is exposed to pure  $\text{N}_2$  on one side, and to room air on the other, there is a chance of a slight error, even though the sample is not actually forced into the KOH. To have the KOH protected from the room air by a bulb of  $\text{N}_2$  would be ideal for the determination of the total absorption. The conditions which prevail in the usual set-up are nearly perfect for the determination of  $\text{CO}_2$ . The simplest procedure seems to be the use of one apparatus for the determination of  $\text{CO}_2$  and another (with the KOH replaced by water) for the determination of total absorption. The one used for total absorption would have  $\text{N}_2$  on each side of the KOH. As indicated above, this is desirable only when a considerable number of samples are at hand.

It should be noted that by ignoring the change in the amount of water on the mercury during an analysis the resulting oxygen value is too high. Putting the sample of nitrogen into the KOH solution at the end of an analysis gives an oxygen percentage which is too low. These two errors, being of the same general magnitude, nearly compensate. This fact, however, hardly justifies ignoring them.

#### THE COMPOSITION AND STORAGE OF OUTDOOR AIR

The following results were obtained on outdoor air by determining both the total absorption and the  $\text{CO}_2$  in duplicate or in triplicate. These data show the extent of uniformity found in the composition of outdoor air.

TABLE III

DATE	TOTAL ABSORPTION	$\text{CO}_2$	O
March 25	20.979	0.036	20.943
March 26	20.977	0.035	20.942
March 29	20.968	0.029	20.939
April 2	20.968	0.030	20.938
April 22	20.974	0.034	20.940
May 4	20.973	0.034	20.939
May 11	20.973	0.035	20.938
May 18	20.974	0.023	20.941
Average	20.973	0.033	20.940

The analysis of outdoor air, therefore, and the determination of  $\text{CO}_2$  and  $\text{O}_2$  (and a respiratory quotient) from burning acetone,<sup>3</sup> serve well in checking the correctness of results of an analysis.

The use of a satisfactory respiratory quotient as a sole criterion in testing the tightness of a metabolism apparatus is inadequate since a leak of room air into it reduces both the apparent  $\text{CO}_2$  production and oxygen consumption, and thus may scarcely at all affect the respiratory quotient.

Samples of outdoor air were taken and stored in glass sampling tubes and analyzed after one week and two weeks to determine the change in composition during storage. These samples contained the water vapor of outside air at the time of sampling. One group (A) was stored in perfectly clean tubes, another (B) in tubes covered on the inside surface with a film of mercury, and a third group (C) contained an appreciable quantity of mercury in the tubes. As stated by Haldane<sup>4</sup> the tubes should be dry and clean. The results are shown in Table IV below, and require no special comment except to point out that the oxygen percentage decreased in the tubes in which samples were stored with even small amounts of mercury.

TABLE IV

DAYS OF STORAGE	A		B		C	
	$\text{O}_2$	$\text{CO}_2$	$\text{O}_2$	$\text{CO}_2$	$\text{O}_2$	$\text{CO}_2$
0	20.939	0.034	20.939	0.034	20.939	0.034
7	20.940	0.035	20.940	0.035	20.920	0.035
14	20.941	0.033	20.922	0.033	20.894	0.029

## SUMMARY

In the analysis of air, it is desirable to determine the  $\text{CO}_2$  and  $\text{O}_2$  together, separate from the determination of  $\text{CO}_2$ , if many samples are to be analyzed.

The protection of the pyro solution from exposure to the air, during introduction into the absorption pipette, materially extends the usefulness of the solution.

To disregard the dehydrating action of the pyro solution causes a plus error in the computed oxygen percentage.

Forcing the sample of nitrogen into the KOH solution, at the completion of an analysis, causes a minus error in the computed oxygen percentage.

Samples of outdoor air stored in tubes containing mercury show a decrease in oxygen.

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## AN AUTOMATIC METHOD OF RECORDING BLOOD FLOW\*

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**F**OLLOWING the discovery of the circulation of blood by Harvey in 1615 many methods have been devised to measure the rate of blood flow. Since the activities (at least in a quantitative sense) of many organs are revealed only by a study of the blood, knowledge of the quantity of blood flowing through an organ is of fundamental importance.

In the main there have been two principal types of apparatus used. One, various modifications of the stromuhr first devised by Ludwig and two, thermoelectric stromuhrs. The older methods are described in Tigerstedt's *Handbuch der Physiologischen Methodik*. Of the modifications of the Ludwig stromuhr, two should be mentioned. Barcroft (1929) built a mechanical stromuhr which appears to be an excellent instrument. More than ordinary mechanical skill, however, is required in fabricating the instrument. Montgomery and Lipscomb (1929) constructed an instrument in which the blood causes a column of mercury to be displaced, which in turn activates a Pavlov electromagnetic valve (1887). It is a satisfactory means of measuring arterial flow but the back pressure developed would certainly limit its usefulness on the venous side of an organ. Gesell and Bronk (1926), Rein (1928), and Herrick and Baldes (1931) have used the principle of the second group, the thermoelectric stromuhr. This method certainly is the most physiologic because the electrodes and thermocouples are approximated to the blood vessel in situ without severing the vessel. However, the method does not measure the flow with great accuracy. Herrick and Baldes after an extensive investigation found it to be reliable only within 10 per cent.

A method has been described by Gayet and Guillaumie (1930). The blood flow is interrupted from its regular course and directed into a measuring chamber of known volume. The time necessary to fill the chamber is noted. Then the original circulation is made and the blood drawn permitted to flow into the animal. The method we believe is satisfactory for approximate work but the human error is difficult to evaluate and the method does not give a continuous measurement.

We have investigated various mechanical stromuhrs and have built one in which many of the objectionable features of others are minimized or eliminated.

### DESCRIPTION OF THE STROMUHR

Fig 1 shows structure and dimensions of the glass stromuhr. Fig 2 shows the construction of the Pavlov valve and the stromuhr with tubes in place. Fig 3 is the wiring diagram of the apparatus which operates the valve.

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## OPERATION

The four platinum electrodes are heavily electroplated with copper. The Y tubes which are connected to the inlet outlet of the stromuhr are crossed so that when the bar of the valve is forward the blood will enter one side and leave by the other. When the bar is pulled back the direction of flow in the Stromuhr is reversed. The balls of the stromuhr are coated inside with a paraffin-wax mixture. The tube "A" (Fig 1) which connects the two cylinders "B" is filled with chloroform just to the level of the electrodes by way of the stopcocks at the top of the stromuhr. An additional amount is then added equal to the desired volume transfer of blood per shift of the valve. A saturated copper sulphate solution is placed in both cylinders "B" to a point about halfway between the upper electrode and

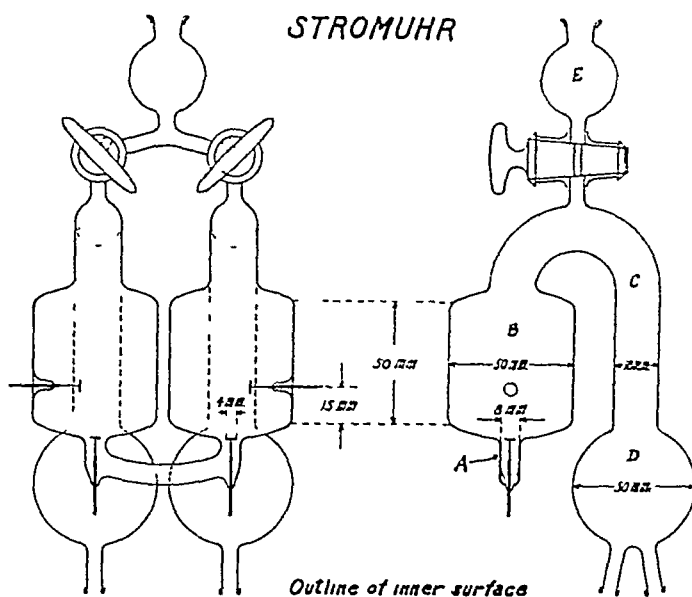


Fig 1

the top of the cylinder. Now place the inlet and outlet tubes from the balls "D" in a beaker of saline and by gentle suction at "E" half fill them with the saline. Then clamp the inlet outlet tubes. Fill the reservoir "E" with a high grade mineral oil of low viscosity (L G White Mineral Oil sp gr 847, viscosity 75-80 Standard Oil Company of Indiana). By intermittent *gentle* suction one can fill the remaining space of "B," "C," and "D" with the oil. Then close the stopcocks. Connect the inlet tube to a reservoir containing saline and the outlet to a measuring flask, and close the switches which connect the stromuhr electrodes with the relays. The apparatus is now ready to be calibrated. Permit fluid to pass through the stromuhr at about the rate of blood flow to be measured until the valve has shifted about 50 times. The volume measured divided by the number of shifts is the shift-volume, the magnitude of which will depend upon the volume of the  $\text{CHCl}_3$  used in the apparatus. There is a small error introduced when the rate of

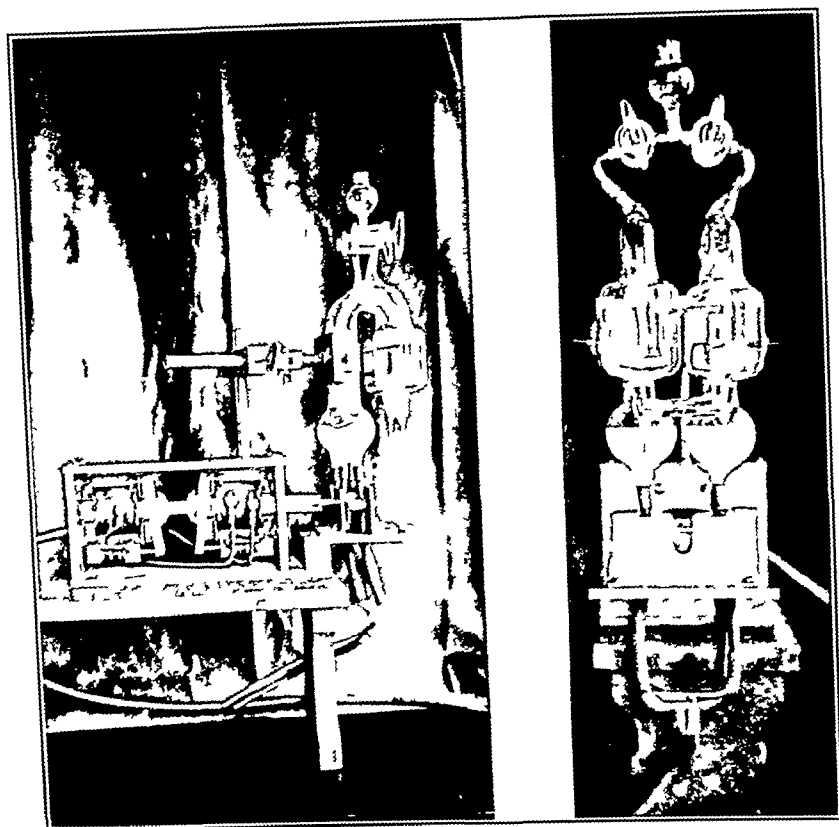
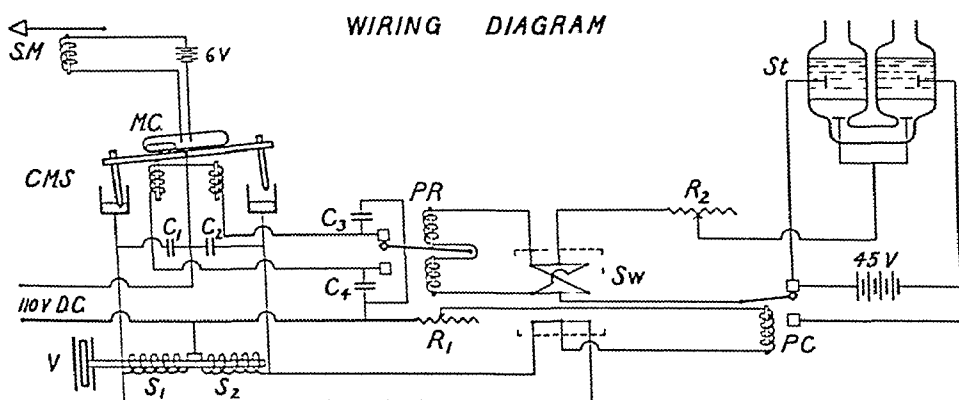


Fig 2



$C_1$  -  $C_4$  2 mf condensers  
 CMS carbon-mercury switch  
 MC mercury contact  
 PC 400 ohm pole changer WUTCo  
 PR 600 ohm polar relay 6-c WUTCo  
 $R_1$   $R_2$  3000 ohm rheostats

$S_1$  push solenoid CR-9503-209 c GEC Co  
 $S_2$  pull solenoid CR-9503-209 GEC Co  
 SM signal magnet  
 Sw triple pole double throw switch  
 St stromuhr  
 V valve

Fig. 2

flow changes Fig 4 shows the error to be less than 1 per cent when the rate of flow is increased 100 per cent over the rate used for calibration

Although the tendency of the electrodes to gas is minimal the use of the pole change (or relay) P C eliminates that objectionable feature In Fig 3 it will be noted that as soon as relay P R closes to operate C M S and S, that P C opens the circuit between the functioning electrodes and connects the battery (with reversed polarity) to the other pair of stromuhr electrodes Thus the current flows between the electrode only momentarily Switch S W is used to reverse the polarity of the battery to the stromuhr and prevent depleting the electrode

We have used heparinized animals in our work One full dose of heparin (15 mg per kilo) is given intravenously just before connecting the stromuhr to the animal One dose per hour is then administered by a constant injection apparatus

### CALIBRATION OF STROMUHR

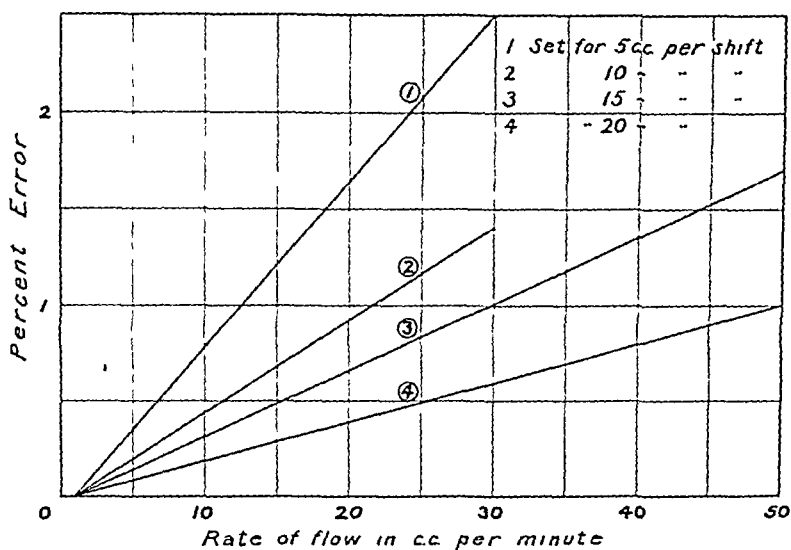


Fig 4

The tubes leading from the animal to the stromuhr should be as short as possible We place the valve and stromuhr on a heavy bracket just above the animal

This apparatus has been used by us to measure blood flow on the venous side of organs with very satisfactory results The parts are neither expensive nor difficult to build It is entirely automatic in operation and the back pressure is less than 7 mm of water

### ADDENDUM

We now use two relays (Struthers Dunn Inc, Philadelphia, type ABYSS) in place of the carbon mercury switch (CMS) The resulting decreased lag reduces the errors charted in Fig 4 to one-third the values given

We wish to express our appreciation to Messrs Rust and Clark of the Western Union Telegraph Company of Chicago for the gift of the relays used in this work

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## A STUDY OF VITALI'S TEST FOR ATROPINE\*

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VITALI'S test is used to characterize the alkaloids, veratrine and atropine. With this test the former gives an orange to reddish violet coloration, whereas the latter gives a deep reddish violet color. The reaction depends upon the oxidation of atropine with concentrated nitric acid and the subsequent formation of an intense reddish violet color† on the addition of alcoholic potassium hydroxide solution. The color after considerable time changes to a cherry-red.

The test was proposed in 1880 by Vitali<sup>1</sup> who tested over 60 alkaloids and found that only 3, daturine, hyoscyamine, and duboisine, gave the violet color.

Witthaus<sup>2</sup> reports that in addition to hyoscyamine, scopolamine gives the test. Van Urk<sup>3</sup> found several alkaloids and other substances which gave similar reactions. Celsi<sup>4</sup> has reported that the positive test is given by the esters of aromatic acids but not by the free acid.

Fuller<sup>5</sup> in dealing with crude drugs found the petroleum ether extract from belladonna, coca, nux vomica, and yohimbe to give a color reaction similar to atropine.

A study of Mandelin's and Frohde's tests for alkaloids in this laboratory<sup>6, 7</sup> has shown that these tests are not specific. Levine<sup>8</sup> and Levine and Magiera<sup>9</sup> have found certain alkaloidal reagents to be more or less general reagents for phenols.

The object of the investigation reported in this paper was to test a large number of organic compounds using Vitali's test, in order to determine whether or not the characteristic violet color was given by any number of the organic compounds. Also, to determine whether or not this coloration was due to any particular organic grouping. The effect of impurities on the test was also studied.

## PROCEDURE

*Preparation of Standard Atropine Solution*—A solution was prepared in alcohol containing one milligram of atropine per cubic centimeter.

*Preparation of Solutions to be Tested*—The compounds to be tested were dis-

\*From the Department of Chemistry, University of Colorado.  
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<sup>7</sup>Most textbooks describe the color as violet.

solved in the proper solvent so that each cubic centimeter was equivalent to one milligram of the compound

*Procedure of Tests*—Three tests were made on each compound. For the first test, one cubic centimeter of the solution to be tested was evaporated to dryness, two or three drops of fuming  $\text{HNO}_3$  were added and again evaporated on a water-bath. After cooling, three drops of alcohol  $\text{KOH}$  were added and any coloration noted. In the second test, one cubic centimeter each of the organic compound and standard atropine solutions were mixed and the test made as described above. If there were no interference a deep violet color should result. The third test was carried out as above, except that five cubic centimeters of the solution of the organic compound were used with one cubic centimeter of the atropine.

The results obtained when Vitali's test was applied to the organic compounds are given in the pages immediately following. The name of the organic compound is given first and directly opposite is given the color reaction. In cases where no color was developed other than that caused by the reagents, the compounds are listed with the statement "No color reaction."

#### ALKALOIDS AND ALKALOIDAL SALTS

ORGANIC SUBSTANCES	COLOR REACTION
Apomorphine	Greenish brown
Atropine	Deep reddish violet
Belladonnine	Deep reddish violet
Berberine	Greenish brown
Cinchonine	Orange
Codeine	Yellowish red
Cokineine	Dark Brown
Cryptopine	Dark Brown
Daturine	Deep reddish violet
Ergotine	Yellowish brown
Homatropine	Yellowish brown
Hydrastine	Yellow
Hyoscyamine	Deep reddish violet
Phenyleine	Reddish brown with violet
Physostygmine	Violet to brown
Quinidine	Brown to violet on heating
Quinine	Orange red
Scopolamine	Deep violet
Strychnine	Yellow
Veratrine	Light rose violet

*No Color Reaction*—Aconitine, brucine, caffeine, emchouidine, cocaine, cotarnine hydrochloride, delphinine, diamine, cinetine, betacucaine hydrochloride, gelsammine, herome, morphine, narcaine, nicotine, papaverine, pelletierine, pilocarpine, piperine, pseudopelletierine, sanguinarine nitrate, sparteine, solanine, theobromine, theophylline

#### AMINO ACIDS AND DERIVATIVES

para-Aminophenylglycine	Brown
Diodotyrosine	Yellowish brown
Glycyltryptophane	Reddish brown
Hippuric acid	Brownish yellow
para-Nitrophenylglycine	Red
alpha-Phenylalanine	Red to brown
Phenylglycine	Reddish brown, trace violet
Tryptophane	Reddish brown
Tyrosine	Yellowish brown

*No Color Reaction*—Acetylphenylglycine, alpha-alanine, arginine, asparagine, aspartic acid, di-benzoylalanine, betaine hydrochloride, creatine, creatinine, edestine, ethylglycollate, glutamic acid, glycine, isoleucine, leucine, beta-phenylalanine, dl-valine



## ALIPHATIC ACIDS

dl alpha Aminoacetylacetic acid  
Mucic acid

Slate  
Brown

*No Color Reaction*—Aconitic acid, adipic acid, alpha bromopropionic acid, beta bromo propionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malic acid, malonic acid, mesaconic acid, palmitic acid, propionic acid, stearic acid, succinic acid, tartaric acid, tartaric acid, trichloroacetic acid

## ALIPHATIC ACID SALTS, ESTERS, AND DERIVATIVES

*No Color Reaction*—Ethyl oxalate, ethyl succinate, isoamyl propionate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate, thallous formate, thallous malonate, triacetin, tributyrin

## ALIPHATIC ALCOHOLS AND KETONES

Methyl heptenone  
Phorone

Brown  
Brown

*No Color Reaction*—Acetylacetone, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isobutyl alcohol, isopropyl alcohol, mannitol, methyl alcohol, octyl alcohol, trichlorobutyl alcohol

## SUGARS

*No Color Reaction*—Arabinose, galactose, glucose, lactose, levulose, maltose, d mannose, melezitose, raffinose, rhamnose, sucrose, xylose

## UREA AND URIC ACID DERIVATIVES

Allylphenylthiocarbamide  
Allylthiocarbamide  
Diphenylthiourea  
Guanine hydrochloride  
Peralga (Aminopyrinediethylbarbiturate)  
Triphenylguanidine

Reddish orange  
Red  
Red  
Reddish orange  
Brownish orange  
Red

*No Color Reaction*—Allylthiourea, amital (isoamylethylbarbituric acid), acetyl methylurea, allantoin, alloxanthine, barbitol (diethylmalonylurea), barbituric acid, biuret, dl n butylthiourea, dibromobarbituric acid, ipral (calcium ethylisopropylbarbiturate), luminal (phenylethylbarbituric acid), thiobarbituric acid, thiourea, urea, urethane, uric acid

## GLUCOSIDES

Amygdalin  
Phlorhizin  
Picrotoxin  
Santonin

Reddish brown  
Dirty green  
Violet  
Reddish brown, trace violet

*No Color Reaction*—Aesculin, arbutin, colocynthin, convallamarin, digitalin, clatrin, salicin, saponin

## MISCELLANEOUS ALIPHATIC COMPOUNDS

Acetamide  
Oenanthol

Red  
Yellowish brown

*No Color Reaction*—Acetal, acetaldoxime, acetoxime, aldehyde ammonia, aminoguanidine bicarbonate, bromotorm, tertbutyl bromide, chitin chloral hydrate, chloral urethane, chloropurine, dimethylloxime, hexachloroethane, hexamethylentetramine, iodotorm, isobutylbromide, methylglyoxal sodium bisulphate, monochlorohydrin, nitrosodimethylamine, oxamide, picric acid hydrate, propionamide sulphonic acid thiolamine, tribromohydrin, trichloroacetylchloride, trimethyl carbamide, triethylamine, xeronol

## BENZENE AND TOLUENE DERIVATIVES

Azobenzene	Violet to brown to black
Mesitylene	Brown

*No Color Reaction*—ortho Bromochlorobenzene, para bromochlorobenzene, ortho bromonitrobenzene, meta chloronitrobenzene, ortho chloronitrobenzene, para chloronitrobenzene, para chlorotoluene, ortho dichlorobenzene, 2,5 dichloronitrobenzene, iodosobenzene, isopropylbenzene, meta nitrotoluene, ortho nitrotoluene, para nitrotoluene, styrene, para xylenesulphonic acid

## ANILINE AND DERIVATIVES

Acetanilide	Yellow to brown
Acetyl ortho methylaniline	Reddish brown
Acetylphenetidine	Brown
Acetyl ortho toluidine	Reddish brown
Acetyl para toluidine	Orange brown
meta Acetylaniline	Brown
Aminoazobenzene	Brown
2 Aminotoluene 4 sulphonic acid	Brownish black
meta Anisidine	Reddish brown
ortho Benzotoluide	Reddish brown
para Bromoacetanilide	Red to brown
meta Bromoaniline	Reddish brown
para Bromoaniline	Reddish brown
meta Chloroaniline	Reddish brown
1, 2, 4 Dinitroaniline	Reddish brown
meta Nitroaniline	Reddish brown with violet
para Nitroaniline	Brown with violet
para Nitrodimethylaniline	Bright red
Nitrosodimethylaniline	Orange red
1, 2, 3, Nitrotoluidine	Brown to black
1, 2, 4 Nitrotoluidine	Brown
1, 3, 4-Nitrotoluidine	Brown to black
ortho Phenetidine	Reddish brown
para Phenetidine	Brown
meta Toluidine	Brown to black
ortho Toluidine	Reddish brown
para Toluidine	Brown
Trinitroaniline	Reddish brown

*No Color Reaction*—Acetyl para anisidine, acetyl para methylaniline, 2 aminotoluene-5 sulphonic acid, 4 aminotoluene 2 sulphonic acid, aniline, ortho anisidine, para anisidine, benzamide, para benzotoluide, ortho bromoaniline, chloramine, ortho chloroaniline, para chloroaniline, 1, 2, 4 dichloroaniline, 2, 5 dichloroaniline, evargine, meta nitrodimethylaniline, phenyl beta diphenylamine, toluidine, tribromoaniline, 1, 2, 4 xylidine, 1, 3, 4 xylidine

## PHENOLS AND DERIVATIVES

Acetyl meta aminophenol	Reddish brown
Acetyl para aminophenol	Greenish brown
meta Aminophenol	Dark brown
ortho Aminophenol	Brown to black
para Aminophenol	Brown to black
para Benzalamminophenol	Dirty brown
ortho Chloromercuriphenol	Greenish brown
ortho Nitrophenol	Brown
Orcinol	Greenish brown
Phloroglucinol	Green
Pyrogallie acid	Brownish green
Tetrabromo ortho phenol	Reddish brown

*No Color Reaction*—5 Benzalamino 2 cresol, benzoylthymol, para bromophenol, carvacrol, catechol, ortho chlorophenol, para chlorophenol, meta cresol, ortho cresol, para cresol, 3, 5 di bromo ortho cresol, 2, 4 dichlorophenol, dimethylhydroresorcinol, 2, 3 dinitrophenol, 2, 4 dinitrophenol, 2, 6 dinitrophenol, meta nitrophenol, para nitrophenol, phenol, picric acid, thymol, tri bromophenol, trichlorophenol, xyleneol

## AROMATIC ACIDS

Acetylsalicylic acid	Orange
di Aminophenylacetic acid	Dark brown
Anisic acid	Violet to rose red
Arsanilic acid	Green
Duodosalicylic acid	Orange
Diphenylacetic acid	Slight violet to brown
5 Iodosalicylic acid	Orange
Metanilic acid	Dark brown
Naphthionic acid	Brown

*No Color Reaction.*—meta Aminobenzoic acid, para aminobenzoic acid, anthranilic acid, benzoic acid, meta bromobenzoic acid, ortho bromobenzoic acid, para bromobenzoic acid, meta chlorobenzoic acid, ortho chlorobenzoic acid, para chlorobenzoic acid, cinchophen, cinnamic acid, coumaric acid, gallic acid, mandelic acid, para mercurichlorobenzoic acid, meta nitrobenzoic acid, ortho nitrobenzoic acid, para nitrobenzoic acid, quinic acid, salicylic acid, tannic acid, terephthalic acid, ortho toluic acid, para toluic acid

## AROMATIC ACID DERIVATIVES

Benzamide	Brown
Methyl cinnamic ester	Gray brown
Methyl salicylate	Yellowish brown
para Nitrobenzoyl chloride	Pink

*No Color Reaction.*—Benzoic anhydride, benzyl benzoate, butyl benzoate, coumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, isoamyl salicylate, isobutyl benzoate, methyl benzoate, neocinchophen (ethyl 6 methyl 2 phenylquinoline 4 carboxylate), nicotinic acid nitrate, meta nitrobenzoyl chloride, phenyl salicylate, phthalimide

## AROMATIC ALDEHYDES, ETHERS, ALCOHOLS, AND KETONES

para Aminoacetophenone	Rose violet to brown
Anisaldehyde	Rose violet to brownish red
Benzalacetophenone	Greenish brown
Benzhydrol	Dark brown
para Nitroanisole	Red orange
5 Nitrosalicylaldehyde	Orange
Salicylaldehyde methyl ether	Red
Saligenin	Reddish brown
Tetramethyldiaminobenzophenone	Red
para Tolylaldehyde	Greenish brown
Vanillin	Reddish brown

*No Color Reaction.*—Anisole, benzalacetone, benzophenone, ortho bromoanisole, para-bromoanisole, ortho bromonitrobenzaldehyde, 1, 2, 5 bromosalicylaldehyde, ortho chlorobenzaldehyde, isophthalaldehyde, meta methoxysalicylaldehyde, methylacetophenone, ortho nitrobenzaldehyde, phenetole, phthalic acid aldehyde, piperonal, salicylaldehyde

## HETEROCYCLIC COMPOUNDS

Acridine	Brown
Antipyrine	Brown to black
Isatin	Reddish brown
Skatole	Brown with trace lavender

*No Color Reaction.*—Dimethylpyrone, furoic acid, 6 nitroquinoline, oxyquinoline, piperidine, quinidine, quinoline, succinimide

## HYDROAROMATIC COMPOUNDS

Carvone	Brown
Citronone	Brown

*No Color Reaction.*—d Borncol, l borncol, dl camphor (natural), camphor (synthetic), camphoric acid, camphorsulphonic acid, limonene, menthol, quercite, terpinol, terpenyl acetate

## NAPHTHYLENE AND ANTHRACENE DERIVATIVES

Acenaphthene	Brown
Acet alpha naphthalide	Dark brown
Acet beta naphthalide	Reddish brown
Alizarin	Brown
1, 5 Dimethylnaphthalene	Reddish brown
Dibromanthracene	Reddish brown
Naphthalic anhydride	Reddish brown
beta Naphthol	Green
alpha Naphthylamine	Slut
beta Naphthylamine	Reddish brown
alpha Naphthylaminobenzene	Dark brown
alpha Naphthylisocyanate	Red
Nitroso beta naphthol	Green

*No Color Reaction* — alpha Bromonaphthalene, beta naphthylsulfonic acid, naphthol methyl ether

## MISCELLANEOUS AROMATIC COMPOUNDS

Amarine	Orange red with violet
Benzocatechin	Grey brown
Benzoin	Dark brown
Benzylphenylhydrazine	Brown
2, 4 Dimethoxyphenylhydrazine	Dirty violet with brown
Fluorene	Green to black
Isoamylphenylhydrazine	Reddish brown, trace violet
para Nitrophenylhydrazine	Violet to blue black
Phenanthrene	Greenish brown
Phenanthrenequinone dioxime	Green to dark brown
Phenylhydrazine hydrochloride	Reddish brown trace violet
Thymolphthalein	Brown to greenish brown
para Toluylisocyanate	Brown, streaks of violet
para Toluylsulfonamide	Grey
Triphenylmethane	Violet to blue
Turmeric	Brown

*No Color Reaction* — Abietic acid, adrenaline, benzil, diphenyl, isocoumarol, ortho nitroacet meta xylidide, meta nitrobenzylhydrazide, phenolphthalein, meta phenylenediamine hydrochloride, quercetin, rheumitine (sidoquinone sulfolate), salvarsan, tetrabromophenolphthalein, thiosemicarbazide, ortho toluenitrile, para toluenitrile, para toluylthioquinone

The color reactions for the tests where an equal amount of impurity was added to the atropine were recorded, and also the reactions where five times the amount of impurity was added, but the listing of these would require too much space. Therefore, only those compounds which caused the violet color to be completely masked will be given. Of the remainder of the compounds, many gave no interference whereas others gave more or less interference.

Organic compounds which completely cover up the Vitali test for atropine when present in equal amounts were

Acet-alpha-naphthalide, acet-beta-naphthalide, acetyl-para anisidine, acetyl-ortho-methyltoluidine, acetylphenetidine, alizarine, allylthiocarbamide, allylthiocarbamide, para-aminoazobenzene, meta aminobenzoic acid, para aminobenzoic acid, para-aminophenol, 2-aminotoluene-4-sulphonic acid, 2-aminotoluene-5-sulphonic acid, benzanilide, para-bromoacetanilide, cumaric acid, edestin, glycyltryptophane, hippuric acid, 5-iodosalicylic acid, isoengiol, beta-naphthol, alpha-naphthylisocyanate, para-nitro dimethylaniline, meta-nitrophenol, ortho-nitrophenol, peralga, phenanthrene, phenanthrene quinone dioxime, para-phenetidine, phenolphthalein, salvarsan, tetrabromophenolphthalein, tetramethyldiaminobenzophenone, trimitroaniline, triphenylguanidine, tryptophane, vanillin

Organic compounds, in addition to those listed above, which completely cover up the Vitali test for atropine when present in amounts five times that of atropine were

Abietic acid, meta-acetylhydine, acetyl-meta-aminophenol, acetyl-para-aminophenol, acetyl-ortho-toluidine, acetyl-para-toluidine, aesculine, amarine, para-aminophenylglycine, 4-amino-toluene-2-sulphonic acid para-anisidine, anthranilic acid apomorphine, asanilic acid, benzalamino-2-cresol, para-benzalaminophenol, benzoin, benzotoluid, alpha-bromonaphthalene, brucine, ortho-chloromercuri-phenol, codeine meta-cresol, cryptopine, diiodosalicylic acid, 1, 5 dinitronaphthalene, 2, 4-dinitrophenol diphenylthiourea, emetine, fluorene, guanine hydrochloride, hydragristine, isatin, isoamylphenylhydrazine, isoamylsalicylate, metanilic acid, morphine, naphthionic acid, alpha-naphthylamine, alpha-naphthylaminoazobenzene, narceine, para-nitrophenol, para-nitrophenylglycine, nitroso-beta-naphthol, 1, 2 3-nitrotoluidine, 1, 2 4-nitrotoluidine, oicmol, ortho-phenetidine, phenylglycine, phloroglucinol, phloridzin, physostigmine, piperine, rheumatine, salicin, salicylaldehydemethylether, santonin, skatole, tetrabromo ortho-cresol, thymol, thymolphthalein, ortho-toluidine ortho-tolunitrile, para-tolunitrile, tribromoaniline, tyrosine, 1, 3, 4-xylidine

From a study of the preceding data, it will be noted that there were twenty-seven organic substances which gave various shades of violet, lavender, or purple, which might, to some degree be confused with the Vitali test for atropine. Van Urk<sup>3</sup> reported the following to give some shade of violet, but our work did not show any violet color in the test phenylglycine, ortho aminophenol, pyrogallie acid, and aesculine

In general, the compounds giving a similar atropine test do not belong to any definite group of organic compounds nor does any special organic radical seem to be responsible for the characteristic test. Nearly all of them, however, contained nitrogen in some form

There were a number of organic compounds which partially covered up the atropine test. Of course, in a carefully conducted analysis, many of these substances would be removed by means of the different organic solvents

#### CONCLUSIONS

1. A large number of organic compounds have been subjected to Vitali's test. Some of these have been found to give a color reaction similar to atropine

2. The interferences of organic compounds when present in varying amounts with Vitali's test for atropine have been determined

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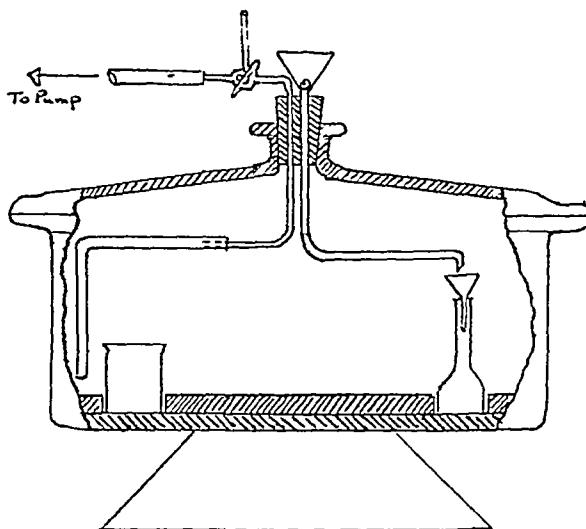
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## A MODIFIED WITT FILTRATION APPARATUS SUITABLE FOR MULTIPLE POTASSIUM DETERMINATIONS<sup>2</sup>

A R McINTYRE, ANN ARBOR, MICH

A SIMPLE modification of the micro-filtration apparatus described by Shohl<sup>1</sup> has been found very useful in the determination of the potassium content of biologic fluids. By means of this modified apparatus multiple determinations of potassium may be made without the necessity for the removal of the upper portion of the chamber thus avoiding repeated reexhaustion of the air from the desiccator.



The apparatus, as modified, consists of a large desiccating jar provided with a tightly fitting rubber stopper bored with two holes. Through one passes the tube connected with the water pump. The distal end of this tube is extended by a short piece of pressure tubing so that upon readmission of air to the apparatus it enters near the base of the chamber. Through the other hole in the rubber stopper is inserted the small glass funnel containing the asbestos mat as described by the above author. The stem of this funnel is bent at right angles and the tip again bent at right angles so as to allow clean drainage. A thick wooden block provided with a series of six holes, each large enough to accommodate the base of a 25 c c volumetric flask and bored so that the holes lie along the circumference of a circle equal in

\*From the Laboratory of Pharmacology, University of Michigan Medical School  
 Received for publication July 9 1932

radius to the length of the horizontal portion of the funnel-stem, is placed on the floor of the desiccator. Two larger holes, also on this circumference, are provided to accommodate beakers for the reception of the excess chloroplatinic acid and the solutions used for washing the filtrate respectively. By rotation of the funnel its stem may be brought over any of the volumetric flasks or either of the beakers as desired. The time thus saved is considerable.

## REFERENCE

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## CONCERNING THE STABILITY OF GLYCERINATED HEMOLYSIN\*

ROBERT A. KILDUFFE, M.D., ATLANTIC CITY, N. J.

IN A previous communication† I reported that antisheep hemolysin to which an equal volume of chemically pure glycerin had been added showed practically no loss in titer after a period of seven years.

The hemolysin in question prepared in 1916, had an original titer of 1:20,000 when titrated in accordance with the method described for use in Kolmer's quantitative complement-fixation test.

During my service with the A. E. F. from 1917 to 1919 the hemolysin was kept in the refrigerator. It was later shipped by freight from Philadelphia to Pittsburgh, thus being exposed to summer temperature for some time, still later again shipped by freight to Los Angeles, also during summer temperature, refrigerated eighteen months and again shipped by freight from Los Angeles to Philadelphia, and finally, to Atlantic City where it was again refrigerated.

At varying intervals the hemolysin has been titrated according to Kolmer's method, with the results tabulated below:

Original titer, 1916	1:20,000
Titer, 1920	1:18,000
Titer, 1923	1:16,000
Titer, 1932	1:20,000

In each instance the titer was read as the smallest quantity of hemolysin giving complete, sparkling hemolysis with 0.5 c.c. of pooled 1:30 complement and 0.5 c.c. of 2 per cent cell suspension.

The last titration was conducted with sheep cells of normal fragility test reaction.

The variations, in view of the last result, were apparently related to variations in complement activity rather than to variations in the hemolysin.

It appears, therefore, that glycerinated hemolysin may be preserved for a period of sixteen years without special precautions as to consistent temperature.

\*From the Laboratories of the Atlantic City Hospital.  
 Received for publication June 23, 1932.  
 †Kilduffe, P. A. A Note Upon the Stability of Preserved (Glycerinated) Antisheep Hemolysin. J. Lab. & Clin. Med. 9: 652, 1924.

## GIEMSA STAIN FOR TISSUE, RAPID METHOD<sup>3†</sup>

W L McNAMARA, M D , HINES, ILL

THE greatest drawback to the use of the Giemsa stain in the preparation of histologic sections is the length of time required. The usual procedure consumes from eight to twelve hours with two or more changes of the staining solution. The following modification is in use at this hospital and the results are found to be as satisfactory as those obtained with the longer procedures.

- 1 Fix tissue in Zenker's fluid forty eight hours or more
- 2 Wash in running water twenty-four hours
- 3 Dehydrate, clear and embed in paraffin
- 4 Cut sections 4 to 6 microns in thickness
- 5 Treat sections with xylol followed by graded alcohols
- 6 Treat with Lugol's solution (5 c c to 100 c c distilled water) for thirty minutes followed by one change of 95 per cent alcohol and one of tap water
- 7 Treat sections for ten minutes in a 0.5 per cent solution of sodium hyposulphite
- 8 Wash well in tap water
- 9 Stain for fifteen minutes in the following solution

Giemsa stain (Stock solution)	10 c c
Acetone C P	10 c c
Methyl Alcohol C P	10 c c
Sol Sodium Carbonate 0.5 per cent	2 M
Distilled Water	100 c c

- 10 Wash rapidly in water and differentiate, using the method employed by Dr. S B Wolbach, as follows

Treat sections individually in two changes of the following solution

Colophonium	15 gm
Acetone	100 c c

Renew the solution as soon as the precipitated colophonium fails to dissolve quickly. Differentiation usually takes place in from fifteen to forty-five seconds.

- 11 Pass sections through a solution of

Acetone	70 c c
Xylol	30 c c

Follow with xylol and mount in oil of cedarwood

<sup>3</sup>From the Clinical Laboratory and Laboratory Center and Cancer Center, Edward Hines  
Jr. Hospital  
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<sup>†</sup>Published under R & P 6969 U. S. Veterans Administration



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## CHOLESTEROL, Blood, In Thyroid Disease Arch Int Med 51 22 1931

The blood cholesterol during fasting was determined as a routine in 505 cases of thyroid disease, along with the pulse, the weight, and the basal metabolic rate.

The lowest average values for blood cholesterol are found in patients in or near thyroid crises.

Auricular fibrillation in toxic goiter is associated with the next lowest average level of cholesterol.

The average cholesterol value in all types of exophthalmic goiter is lower than in toxic adenomatous goiter.

Recurrent hyperthyroidism is associated with cholesterol values that are almost as low as those in exophthalmic goiter.

The average cholesterol value in nontoxic goiter is normal although the scatter is wider than expected. Age appears to raise the blood cholesterol in these cases.

Chronic thyroiditis is associated with higher average values than in any other thyroid disease except myxedema.

The level of the blood cholesterol and the basal metabolic rate bear a reciprocal relationship when judged by average values.

## SEMINAL STAINS, Modification of Barberio's Test, Harrison, G. A. Lancet 2 940, 1932

A portion (1 by 1 cm.) of material containing the stain is pushed into a conical centrifuge tube, 1 cc. of 2.5 per cent solution of trichloroacetic acid added and the tube well shaken at intervals. After standing for one hour, the tube is centrifuged and the supernatant fluid discarded. An equal volume of saturated solution of trinitrophenol is then added when, in the presence of spermine, a precipitate forms. The tube is then placed in a boiling water bath until solution occurs and allowed to cool slowly in the bath. When cool, centrifuge if necessary and examine the crystals microscopically. The presence of crystals indicates a positive reaction, amorphous particles are without significance. A copious amorphous deposit may be due to a high concentration of spermine picrate. In this case dilute with one or more volumes of an equal mixture of 2.5 per cent solution of trichloroacetic acid and saturated solution of trinitrophenol and heat and cool as before.

## CARBON MONOXIDE "Normal" Content of Blood, Gettler, A. O., and Mattice, M. R. J. A. M. A. 100 92, 1933

The average content of carbon monoxide in the blood of eighteen persons living in New York City under conditions of minimal exposure was found to be 0.27 volumes per cent. This represents about 1.0 to 1.5 per cent of the hemoglobin combined with carbon monoxide.

The average content of carbon monoxide in the blood of twelve persons confined to a state institution in an ideal rural locality was found to be 0.24 volumes per cent. Most of these showed a hemoglobin saturation of less than 1 per cent.

The average content of carbon monoxide in the blood of twelve New York City street cleaners was found to be 0.69 volumes per cent. This represents about 3 per cent saturation of the hemoglobin with carbon monoxide.

Two taxicab drivers were found to have on several occasions a carbon monoxide content ranging from 1.47 to 4.3 volumes per cent. This represents a hemoglobin saturation of 8.0 to 19.0 per cent.

Tobacco smoking appreciably increases the carbon monoxide in the blood and cannot be ignored in the interpretation of laboratory results

#### **B TUBERCULOSIS Concentration Method For, Frigimelica, C Polielmeco 39 1776, 1932.**

The sputum is first shaken with a few centimeters of water in a beaker and then sufficient saturated aqueous solution of trinitrophenol added to saturate the specimen

The mixture is then stirred with a glass rod until it is thoroughly stained a bright yellow

Then, with gentle heat, add 10 per cent sodium hydroxide until the sputum dissolves. Cool and add one third volume of chloroform, shake vigorously and centrifuge ten minutes. The bacilli are concentrated in the whitish ring formed at the zone of separation of the chloroform

#### **HEMOGLOBIN, Standards, Haden, R L Am J Clin Path 3 85, 1933**

From a thorough survey the author concludes that

In making hemoglobin determinations only an instrument or method should be employed in which the results are recorded in grams as determined by the oxygen capacity or iron content method

For clinical purposes the reports of hemoglobin are best given in percentage of normal of a healthy adult with a red cell count of 5,000,000 cells

The Haldane and Williamson standards can not be so transposed since in determining the standards no red cell counts were done

There is a wide variation in hemoglobin and erythrocyte counts and consequently in the hemoglobin coefficient in the three large series (Haden, Osgood and Haskins, and Wintrobe) reported for normal men and women

A satisfactory standard must give a color index within normal limits (0.90 to 1.10) in normal individuals

In the present state of confusion the hemoglobin coefficient should be determined for each laboratory and the hemoglobin should be reported only in percentage of this normal

The hemoglobin percentage of a given blood when determined by this method is necessarily the same in all laboratories within the limits of technical error

#### **BLOOD CHEMISTRY Comparative Value of Monochlorobenzene and Thymol When Used With Fluoride as Preservatives of Blood For Chemical Analysis, Lewis, R C, and Mills, G C Am J Clin Path 3 17, 1933**

The following is a summary of the results of this study

A combination of monochlorobenzene and potassium fluoride was found to be a more effective preservative for blood sugar, total nonprotein nitrogen, and urea than thymol and potassium fluoride. Little change occurs in the uric acid and the creatinine with either preservative combination

Blood specimens may be transported through the mails in journeys up to eight days with no appreciable change in either the blood sugar or the nitrogenous blood chemical constituents when a preservative combination composed of 0.275 gm of potassium fluoride and 0.2 gm of monochlorobenzene per 20 c.c. of blood is employed. In journeys of twelve days, the sugar is well preserved, and only slight changes occur in the nonprotein nitrogenous constituents

Neither monochlorobenzene potassium fluoride nor thymol potassium fluoride will satisfactorily preserve blood specimens which are subjected to a temperature of 37°C for twenty-four hours or longer

If a blood specimen treated with monochlorobenzene potassium fluoride has been subjected to high temperatures during passage in the mails, there will be a failure of preservation of the blood chemical constituents. Such blood will contain numerous small clots which will interfere with pipetting of the blood. The absence of such clots, therefore, offers a simple criterion as to successful preservation

When preserved by the addition of 0.2 gm of monochlorobenzene to 20 c.c. of blood,

oxalated blood specimens may be stored in a refrigerator at 6°C for ninety six hours with practically no variation in the blood chemical constituents

**ANTIGENS, Flask for Dilution of, Hinton, W. A.** *Am J Clin Path* 3 41, 1933

In view of the necessity for accurate dilution of antigens and the impossibility of eliminating the personal equation in the preparation of antigen suspensions, the author suggests that the dilutions be made in specially prepared flasks

An inverted V shaped partition is blown in the bottom of an ordinary Erlenmeyer flask producing two semicircular compartments. In 25 to 150 c.c. flasks the compartments hold from 3 to 5 c.c. and from 8 to 10 c.c. in the larger sizes (125 to 500 c.c.)

The antigen is pipetted into one compartment and the diluent into the other, mixture is made by shaking the flask quickly from side to side

**TUBERCULIN TESTS Results With Different, Friedman, E., Black, M. H., and Esserman, A. L.** *Am J Dis Child* 45 58, 1933

A study of the various diagnostic tuberculin cutaneous tests, performed with scrupulous regard for details, demonstrated the utter unreliability of the Pirquet and the modified Moro tests

It failed to substantiate the claims advanced by other observers for the "multiple puncture" method

This study demonstrated the superiority of the Mantoux and "single puncture" technique. The recommendation appears warranted that these two tests supersede the others

For routine office and bedside work the "single puncture method" may be given precedence, because it is easier to perform, is less time consuming, eliminates the necessity for dilution and refrigeration, is less expensive and, withal, yields results as accurate as those obtained by the well established Mantoux test

**MENINGITIS, Tuberculous, Laboratory Diagnosis of, Foard, A. G., and Forsyth, A.** *Am J Clin Path* 3 45, 1933

This study is thus summarized

In the diagnosis of tuberculous meningitis from cerebrospinal fluid, the findings of tubercle bacilli on smear or culture or the production of tuberculosis in a guinea pig injected with the fluid is conclusive evidence of the disease. However, by the last two means the diagnosis can rarely, if ever, be made before the patient is dead. Smear examination if done on repeated samples should reveal bacilli in 80 to 100 per cent, depending on the technique and persistence of the examiner. But in the absence of a positive smear the following findings, especially when they are all present (and they usually are), should warrant the tentative diagnosis of tuberculous meningitis, (1) increased cell count, averaging about 200, producing a slight ground glass appearance in the fluid, (2) Differential count usually shows marked preponderance of lymphocytes but may show a moderate number of polys in smear negative cases, (3) Strong tests for globulin (2 to 4), (4) the formation of an inverted pine tree web on standing for several hours or overnight, (5) colloidal gold curve showing maximum precipitation in the sixth or seventh tube, (6) the sugar content markedly or moderately reduced, averaging about 36 mg per 100 c.c. fluid, and (7) a decrease in chlorides below 650 mg. Especially should the association of a marked decrease in sugar accompanied with a noteworthy decrease in chlorides in fluids not showing characteristics of a suppurative meningitis be stressed as a strong presumptive sign of tuberculous meningitis

**INTESTINAL INFECTIONS, In Infants, Johnston, M. A., Brown, A., Tisdall, F. F., and Fraser, D. T.** *Am J Dis Child* 45 1, 1933

To test the validity of the various hypotheses as to the cause of acute intestinal intoxication, 172 patients with acute intestinal intoxication or related diagnoses and 107 controls were subjected to clinical investigation as well as bacteriologic survey of the flora of the upper respiratory tract and gastrointestinal tract. Although evidence of late involvement of the middle ear developed in 63 per cent of the cases, there was similar involvement

in over 60 per cent of the controls who showed no evidence of acute intestinal intoxication. Hemolytic streptococci were isolated very rarely from the ear, nose, and throat in patients either during life or post mortem. Hemolytic streptococci were isolated more frequently from controls both during life and post mortem, although these showed no symptoms suggestive of acute intestinal intoxication.

Evidence was found bacteriologically or serologically of infection with pathogenic microorganisms of the colon paratyphoid dysentery group.

It was concluded that acute intestinal intoxication is not caused by masked mastoid infection.

Acute intestinal intoxication, fermentative diarrhea, infectious diarrhea, dysentery and summer diarrhea are synonyms indicating one condition, a gastro intestinal infection with species of the colon paratyphoid dysentery group or bacilli.

**LIVER FUNCTION, Rose Bengal Test of,** Stowe, W P, Delprat, G D, and Weeks, A. *Am J Clin Path* 3 35, 1933.

The following results are reported:

Normal values (17 cases) 85 to 110 per cent.

Gall bladder disease—rose bengal elimination was in inverse relationship to the acuteness of the disease.

In three normal pregnancies the function varied from 85 to 100 per cent, early pre-eclamptic toxemia (1) 80 per cent, severe eclampsia (1) 55 per cent, hyperemesis gravidarum (2) 55 and 40 per cent.

Cirrhotics gave uniformly low values.

Chronic alcoholism (4) 100, 80, 90, and 42 per cent.

Carcinoma, secondary (2) 78, 80 and 40 per cent.

Diffuse hepatic damage (3) 60, 67, and 57 per cent.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughan, Professional Building, Richmond, Va

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### Some Factors in the Localization of Disease in the Body

**A**MONG the many and varied phenomena of disease, those concerned with the localization of pathologic processes within the body present a problem, as yet unsolved, of great as well as practical interest.

As stated in the introductory chapter of this book "When aggressive agents have entered the body the immediate safety of the individual usually is ensured if the aggressors can be re-tained or collected into a circumscribed region where the integrity of the tissues is not a vital necessity, and where the powers of resistance may be exercised in concentrated force."

The purpose of this book is "to elucidate the circumstances in which such segregation and retention come about, and to determine by what artificial means these defensive processes may be assisted."

It is obvious that this is, indeed, a complex problem not to be easily solved and that to its ultimate elucidation must be bent the work of many investigators in varied fields.

The author has made a distinct and important contribution based upon extensive experimental studies and a careful review and discussion of the literature germane to the subject.

As a basis for his discussion he has studied in detail the localization of colloids, larger organic particles, viruses, and micro organisms from the blood stream and upon these studies has founded the general principles he advances.

For the commonly used phrase, "increased permeability of the blood vessels" he substitutes a new word, "diaporesis," coined by D'Arcy Power and signifying the passage of colloidal or other particles of suspended matter, including bacteria or blood cells, through the unbroken walls of the blood vessels.

His studies of inflammatory processes may be thus summarized:

- 1 Inflammation is the common, though not the only, cause of increased endothelial permeability.

- 2 The essential feature of inflammation is an increased permeability of the injured cell.

- 3 The secondary manifestations of inflammation as seen in the higher animals vary widely with the duration, degree, and nature of the irritant.

- 4 A cell, though surviving an injury, does not always recover completely, it may remain in a condition of abnormal permeability.

- 5 Diapedesis is a passive process, being independent of activity on the part of the leucocytes.

- 6 Not only blood cells, but most particles also, are transported through the capillary endothelium under the influence of inflammation.

The transudation of normal proteins is rendered possible by an increased permeability of the vascular endothelium, to which a number of secondary factors contribute influencing the rate of transudation. Among these are hyperemia, an increased hydrostatic intracapillary pressure, osmotic pressure, diffusion currents, and electrophoresis.

The factors concerned in the production of the capillary endothelium are thus summarized: inflammation, toxins, and accumulation of metabolites, increased  $P_H$  of the blood or tissues, poisons present in the circulating blood, possibly quantitative defects in the constitution of the

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\*Some Factors in the Localization of Disease in the Body. By Harold Burrows. Later Hunt-Ryan Professor Royal College of Surgeons. Cloth. 233 pages 6 figures 3 colored plates. Wm. Wood and Co. New York.

blood, certain cellular degenerations, increased physiologic activity, perhaps an immaturity of the vascular endothelium, and possibly an imperfect recovery of endothelium following injury

As a result of his investigations Burrows concludes that for the localization of many blood borne diseases three conditions are required: an abnormal permeability of the small blood vessels, the presence of forces which will transport the noxious agents through the endothelial cytoplasm, and the retention of noxious agents in the tissues under the influence of inflammation, all of which lead to the localization, not only of the agents of disease, but to the factors of defense

This volume represents a noteworthy contribution to the study of disease and well repays perusal and study

## Endocrine Medicine

BECAUSE of an ever increasing knowledge of the functions of the various endocrine glands, it may be conservatively said that this speciality is now looked upon as an integral and indispensable part of modern medicine. Interest in this subject has been widespread as well as intensive, and as a result fundamental discoveries have followed one another in rapid succession during the past fifteen years. Naturally there has been considerable interspersed conjecture and theorizing, and much has been written that was later disproved or discarded.

Engelbach's "Endocrine Medicine" is an up to date, conservative but comprehensive appraisal of the known facts concerning the endocrine glands. This work comprises three volumes. The author has approached his treatise in a logical, sequential and natural manner, replacing dogmatism with known facts, and thereby treating the reader to a freedom of thought that is both healthy and refreshing.

The first volume concerns itself for the most part with fundamentals. There is a fairly comprehensive account of the history of endocrinology in the opening chapters. Organology, physiology, nomenclature and etiology are adequately covered, and diagnostic procedures are thoroughly discussed. The relation of the endocrinopathies to general medicine, the specialties and to public health is frankly considered.

The second volume is taken up with a thorough consideration of the infantile and juvenile endocrinopathies.

Adolescent and adult endocrine disturbances are treated, in the third volume, with a painstaking and meticulous care for detail characteristic of the entire work. This volume alone contains 862 pages.

There is a foreword by Dr. Lewellys F. Barker. The entire work is profusely illustrated, and there are many helpful and interesting charts as well.

It is quite evident that the author was a master of his subject but in no instance has zealousness been substituted for fact.

This should be an ideal source of information or a reference work for either the student, the specialist, or the internist.

## Skin Diseases and Nutrition†

THE German edition of this book was reviewed in the November, 1932, number of the Journal. For those who cannot read German, the English translation which is now available is excellent.

\*Endocrine Medicine. By William Engelbach, M.D., F.A.C.P., B.S., M.S., D.Sc., Professor of Clinical Medicine, St. Louis University School of Medicine, 1911-24; Physician-in-Chief, St. John's Hospital, 1909-24; Member of Staff, St. Louis City Jewish Baptist Sanitarium and Maternity Hospitals; President of Association for Study of Internal Secretions, 1922-23; President of the St. Louis Medical Society, 1918; Fellow of American Medical Association and American College of Physicians; Member Missouri, Illinois, New York and Southern Medical Societies. With a Foreword by Lewellys F. Barker, Professor Emeritus of Medicine, The Johns Hopkins University School of Medicine. Three volumes and an index volume with 933 illustrations. Volume I: General Considerations. Volume II: The Infantile Endocrinopathies. The Juvenile Endocrinopathies. Volume III: The Adolescent Endocrinopathies. The Adult Endocrinopathies. Springfield, Ill.: Charles C. Thomas, Baltimore, Md., 1932.

†Skin and Nutrition Including the Dermatoses of Children. By Erich Urbach, M.D. Authorized English Translation by Frederick Rehm Schmidt, A.B., M.D. With 55 illustrations, 8 diagrams and 10 tables. Cloth. Pages 242. Wilhelm Maudrich, Publisher, Vienna, 1932.

### The Renal Lesion in Bright's Disease

THE kidney has been one of the chief stumbling blocks for the morphologic pathologist. After nearly a hundred years from the first description of the malady by Bright there is still no unanimity concerning the character of the local lesion, or general agreement in clinical classification. The dictum "conclusions drawn from the urine are as brittle as the urinal" had its basis in careful observation and most of those interested in the pathology of Bright's Disease have long since discontinued efforts to prognosticate the character of the lesion from the study of the urine. Casts may be very numerous at one time and few or absent at another time even though in the latter instance the disease may have progressed seriously.

Addis, realizing that the reason for this variability was not in the kidneys but in variations in the concentration of the urine, such as hyaline casts dissolved in a very dilute or alkaline urine, inaugurated a quantitative study of the cellular elements of the urine.

By taking precautionary measures to provide a relatively concentrated, acid urine, he has been able to make reliable quantitative microscopic observations on twelve hour urine specimens. Space will permit only a very brief mention of his many interesting observations. He found that in twelve hour night collections made with the precautions mentioned, the normal cast excretion ranged up to 5000 per twelve hours. The normal average erythrocyte excretion was 65,750, leucocytes and epithelial cells, 322,550.

Years ago Sir James Mackenzie urged the desirability of studying individuals over years of life and correlating the clinical observations with autopsy findings. This monograph represents an achievement of this sort. Addis has followed his patients over periods up to ten years, making very careful life studies and Oliver has provided the morphologic postmortem observations. The monograph consists in an effort to correlate the two.

The authors produce very satisfactory evidence in support of their contention that conclusions drawn from the urine when the urine is properly examined may be of distinct diagnostic significance. They divide clinical Bright's Disease into three varieties, hemorrhagic, degenerative and arteriosclerotic. The pathologic findings in the kidney are classified as glomerulitis, parenchymal degeneration, interstitial proliferation and arteriosclerosis or endarteritis.

Briefly, in hemorrhagic Bright's Disease there is always a glomerulitis at autopsy. There may or may not be any or all of the other three pathologic lesions. In degenerative and arteriosclerotic Bright's Disease, there is no glomerulitis. In the degenerative form there is always parenchymal degeneration and may or may not be interstitial proliferation and sclerosis. In the arteriosclerotic form there is always interstitial proliferation and arteriosclerosis which may or may not be accompanied by parenchymal degeneration.

The authors prefer the term Bright's Disease to nephritis and nephrosis, feeling that in our present unsatisfactory classification of the pathologic lesion, the original term is less descriptively binding and therefore more desirable.

The monograph contains a wealth of interesting detail.

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### Handbook of Bacteriology†

THIS, the third edition of a well known manual, has undergone a thorough revision throughout and presents the subject in a thorough manner and in conformity with the latest advances in the field of modern bacteriology.

While primarily intended for the use of students this book well deserves, like its predecessors, a place in the reference library of laboratory workers as well as physicians in general.

It can be highly recommended.

\*The Renal Lesion in Bright's Disease. By Thomas Addis, Professor of Medicine, Stanford University, and Jean Oliver, Professor of Pathology, Long Island College of Medicine, formerly Professor of Pathology, Stanford University. With 170 full-page plates, 2 in color, 21 text illustrations and one folding table, cloth, pages 628. Paul B. Hoeber, Inc., New York, 1931.

†Handbook of Bacteriology. By J. W. Bigger, Professor of Bacteriology and Preventive Medicine, University of Dublin, etc. Cloth, 459 pages, 84 figures. William Wood and Co., New York.

## Manual of Clinical and Laboratory Technic

**T**HIS small book is intended for the practical guidance of the student and interne in the systematic study of patients

Only such technical details as properly fall within their field are given in a succinct manner. That the volume has reached a fourth edition is an evidence of its practical usefulness.

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### Aids to Bacteriology†

**T**HIS is one of a series "Student Aids," small, compact volumes, presenting succinct yet comprehensive and practical surveys of specific subjects.

The present edition has been extensively and thoroughly revised bringing the book in line with the newer developments in the field of bacteriology.

While the discussions are necessarily brief the book supplies a satisfactory survey of the subject.

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### P<sub>H</sub> and Its Practical Application‡

**A**SSAID by the authors in their introductory chapter, although the determination of hydrogen ion concentration first became the subject of investigation nearly half a century ago, it is only within the last ten or twelve years that it has become of general interest and application, a natural development arising from the evolution of relatively simple and practical methods now familiar to and utilized by laboratory and technical workers in a variety of fields.

This book is not intended to serve as a manual of technic, but rather as a handbook for the practical technologist who desires a working knowledge of the practical applications of P<sub>H</sub> measurements.

That this purpose is achieved, and that the book, though small, is comprehensive in character is indicated by the following summary of its contents.

The first section of five chapters comprises a discussion of the mechanism of hydrogen ion determinations and, despite the inherent complexities of the subject, is clear, readable, and understandable.

The second section, of fourteen chapters, discusses and illustrates the practical application of P<sub>H</sub> determinations in many fields such as the regulation of water supplies, water corrosion problems, disposal of sewage and industrial waste, sugar industry, gelatin and glue, leather manufacture, textile industries, cleaning processes, electroplating, general industrial chemistry, bacteriology, pathology, and titration procedures, and soils.

The volume is well printed and thoroughly indexed and can be recommended to those interested in the many and diverse problems discussed.

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### Handbook for Senior Nurses and Midwives§

**T**HIS, the second edition of a manual first introduced in 1926, has been extensively revised and includes four new chapters.

The book is divided into five sections: I, Medical; II, Surgical; III, Children; IV, Obstetrical; and V, Gynecological.

The volume is addressed more to the graduate nurse and experienced midwife than to the student and, consequently, discusses the subjects covered in some detail.

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\*Manual of Clinical and Laboratory Technic. By Herman B. Weiss, Associate Professor of Medicine, University of Cincinnati, and Raphael Isaacs, Associate Professor of Medicine, University of Michigan. Fourth Edition. Pp. 117. Pages with Diet Table. Cloth. W. B. Saunders Co., Philadelphia and London, 1932.

†Aids to Bacteriology. By Wm. Partridge. Public Analyst, County of Dorset, etc. Cloth. 111 pages. Fifth edition. William Wood and Co., New York.

‡P<sub>H</sub> and Its Practical Application. By Frank P. LaMotte, William P. Kenny, and Allen B. Reed. Cloth. 255 pages. 18 charts and numerous tables. Williams and Wilkins Co., Baltimore, Md.

§Handbook for Senior Nurses and Midwives. By J. K. Witson, Late House Surgeon, Essex and Colchester Hospital. Cloth. 676 pages. 297 figures. Oxford University Press.



### The Pathogenic Streptococci

THIS volume VII of the Annals of the Pickett Thomson Research Laboratories, is a continuation of the studies presented in preceding volumes of the series, of the role of the streptococcus group in the production of specific diseases

In this book the role of the streptococci in erysipelas, skin diseases, and measles are presented in comprehensive detail embodying an extensive survey of the literature, which, in itself, is of great value

The accompanying microphotographs as usual are excellent and excellently reproduced

This book, like its predecessors, will be received with great interest by bacteriologists and laboratory workers and constitutes a distinct and important contribution to an, as yet, not entirely settled subject

### The Anatomy of the Human Orbit and Accessory Organs of Vision†

THE first edition of this work published in 1921 was a valuable book and, so far as this reviewer knows, the only one of its kind in the English language

This timely second edition is enriched by exactly that amount of knowledge which the world has contributed to the subject in the past eleven years, for as the author modestly says, "over 190 papers published since 1921 have been read and incorporated" The general plan of the book is not altered, but the new material falling in its proper place makes a thorough revision, while adding but 39 pages to the volume

Seventeen illustrations, some in colors, have been added Another pleasing feature is the increased number of paragraph titles in bold faced type which, in effect, serve as an index as one turns the pages The conventional index is in the usual location

The book deserves nothing but praise The first edition was a good friend, much used and loaned, a necessary item in every ophthalmologist's library

### Manual of Surgery‡

THIS, the eighth edition of a well known manual represents the position of present day surgery and in particular the teaching of the Edinburgh School

It will undoubtedly receive the same favorable reception accorded to its predecessors

### Protoplasmic Action and Nervous Action§

THIS second edition, materials and bibliography are brought up to date and cognizance taken of recent developments in this field

The book, largely based upon lectures delivered in Clark University and in the Marine Biological Laboratory, is concerned with the phenomena of reactivity and response in living matter and is essentially a treatise on the physicochemical basis of the more general properties of living matter

It may be regarded as a well planned and well executed exposition of the subject by a prominent worker in this field

\*The Pathogenic Streptococci The Role of the Streptococcus in Erysipelas Skin Diseases and Measles Annals of The Pickett-Thomson Research Laboratory Vol VII Paper III pages 35 microphotographic plates Williams and Wilkins Co Baltimore Md

†The Anatomy of the Human Orbit and Accessory Organs of Vision By S E Whitman Professor of Anatomy McGill University Montreal Second edition Cloth 467 pages 212 figures Oxford University Press

‡Manual of Surgery By Alexander Miles Consulting Surgeon Royal Infirmary Edinburgh and D P D Wylie Professor of Surgery University of Edinburgh Cloth eighth edition 574 pages 176 figures Oxford University Press

§Protoplasmic Action and Nervous Action By I S Lillie Professor of Physiology University of Chicago Cloth 417 pages Second edition University of Chicago Press

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## EDITORIAL

### The Blood Serum Proteins

THE Kjeldahl<sup>1</sup> was until recent years the only method available for the quantitative determination of the total serum protein content or the fractions of albumin, globulin, fibrinogen, etc, separated by the use of 21.5 per cent sodium sulphate at 37° C<sup>2</sup> or saturated solution at 33° C<sup>3</sup> or ammonium sulphate<sup>4</sup>. Clinical investigations were consequently few and far from complete. The Kjeldahl still remains the standard method and the court of last appeal, but the introduction of a much simpler, albeit less accurate, colorimetric method by Greenberg<sup>5</sup> stimulated clinical interest and investigation of the serum protein fractions. Exton<sup>6</sup> has developed a nephometer with photoelectric cells for the rapid estimation of the blood proteins according to the turbidity produced, and most recently Page and Van Slyke<sup>7</sup> have advocated a rapid way of determining whether the proteins are below the edema level.

Christian<sup>8</sup> in the Billing's lecture of a year ago discussed the clinical significance of the blood proteins, especially their osmotic pressure value versus the intracapillary pressure in the production of edema, stressing the relation of oligoproteinemia to the accumulation of edema in renal disease

The importance of the loss of quantities of serum albumin and globulin through the kidneys has long been recognized and Epstein's<sup>9</sup> high protein diet, in order to make up for this loss and to maintain the blood protein levels and overcome obstinate edema, was the first step in the rational management of such cases and the first application of Stirling's<sup>10</sup> conceptions of the function of blood serum proteins

The so called "nephrosis syndrome" is the striking example of the effect of a depleted blood serum as a result of a high grade albuminuria. The albumin fraction is of smaller molecular size and is chiefly lost, there is apparently an attempt at compensation or an increase in the globulin fraction but this Govaerts<sup>11</sup> has shown to be of much less of an osmotic pressure or oncotic value and consequently the hydrophilic power of the blood serum is decreased

Govaerts<sup>11</sup> has estimated that a one gram per cent solution of albumin in serum exerts an osmotic pressure of 5.5 mm Hg (74.8 mm H<sub>2</sub>O) while that of a one gram per cent of globulin in serum is only 1.4 mm Hg (19 mm H<sub>2</sub>O). Normal human serum contains about 4.5 gm of albumin and 2.5 gm of globulin, a total serum protein of 7 gm which exerts an oncotic pressure of about 28 mm Hg (380 mm H<sub>2</sub>O). Krogh,<sup>12</sup> Starling had suggested the existence of an equilibrium of this protein osmotic pressure and the intravascular pressure as early as 1896

The blood proteins are significant in other conditions and may be lost as Turner<sup>13</sup> has pointed out, through other channels, as through the damaged atrophic gastrointestinal tract in pellagra. In pellagra without or with edema, however, as it may also be in wet beri-beri and in waterlogged adult scurvy (a case of which we recently found to have oligoproteinemia) and, as reported by Youmans,<sup>14</sup> in chronic undernutritional states due to diets insufficient in proteins and especially protein of good biologic value, the oligoproteinemia may be the result of an inadequate protein ingestion or assimilation

Absorption of digested protein may be interfered with by the chronic passive congestion of the mucosa of the gastrointestinal canal, but in congestive heart failure there may be as was suggested<sup>15</sup> another factor responsible, namely, an interference with the elaboration of blood proteins in the engorged liver. This is, of course, merely a surmise, depending upon the fact that it seems established that the fibrinogen fraction is formed in the liver. In congestive heart failure,<sup>16</sup> the edema is due primarily to stasis, and oligoproteinemia plays only a minor but sometimes a significant rôle in the exaggeration and persistence

Significant diagnostic and prognostic serum albumin-globulin relations in liver disease with ascites and edema have been reported by Signorelli,<sup>16</sup> but the most striking clinical oligoproteinemias are encountered in the nephrotic syndrome. Leiter, Alexis Hartmann and his coworkers<sup>17</sup> have recently reported favorable diuresis from injections of acacia in chronic resistantly edematous nephrotic children with oligoproteinemia. They were able to raise the oncotic pressure to normal with the colloid solution. This interesting and important field of the blood serum proteins has been made accessible for clinical investigation by modern methods.<sup>18, 19, 20</sup>

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# *The Journal of Laboratory and Clinical Medicine*

VOL XVIII

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No 8

## *CLINICAL AND EXPERIMENTAL*

### MULTIPLE PRIMARY MALIGNANT NEOPLASMS\*†

HOLCOMBE H. HURT, M.D., AND ALBERT C. BRODERS, M.D., ROCHESTER, MINN.

THIS study of the occurrence of more than one primary malignant newgrowth afflicting the same person is based on the records of 2,124 patients with malignant tumors treated at The Mayo Clinic in the calendar year 1929. Only cases in which there was a proved microscopic diagnosis of at least one malignant newgrowth while the patient was at the clinic in the course of 1929, were included in the 2,124.

This study was undertaken primarily to determine the relationship of the grade of malignancy to the frequency of occurrence of multiple primary malignant neoplasms. Broders' system of grading malignant neoplasms was used. The age, sex and family history of the patients were included.

The literature on multiple primary malignant tumors has grown to be extensive since Billroth reported the first case in 1869. Hanlon, working at The Mayo Clinic, reviewed the postulates established by Billroth for determining multiple primary malignancy and showed that they are impossible to fulfill in a large percentage of cases of true and undisputed primary multiple malignant neoplasms.

The criteria of Goetze for diagnosis of multiple primary malignant neoplasms, as given by Hanlon, are reasonable and will be followed in this study. They are (1) the tumors must have the microscopic and macroscopic appearance of the usual tumors of the organs involved, (2) exclusion of metastasis must be certain, and (3) diagnosis may be confirmed by the character of the individual metastasis. Hanlon thoroughly reviewed the literature and reported 48 cases. He concluded that double primary carcinomas are probably incidental.

The work of some investigators, which Hanlon did not have occasion to mention in the published abstract of his thesis is relevant here. Orr expressed the belief that accurate record of the occurrence of multiple malignant neoplasms may decide the disputed question as to whether one

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†This material was used in a thesis submitted by Dr. Hurt to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

malignant neoplasm exercises an inhibitory effect on the development of a second. His figures are insufficient to settle that question, but they tend to show that the incidence of multiple primary malignant neoplasms is about what would be expected, if the occurrence were accidental.

Seechurn, in commenting on the incidence of multiple primary malignant growths, was inclined to believe, with Ewing, that the "rather common occurrence of two or more tumors in different or the same organs of the same subject, suggests nothing more than an accidental coincidence, in several organs, of the general biological factors in the genesis of tumors." But Ewing also suggested that "when minute search is carefully conducted and all forms of tumor growth included, the proportion of multiple tumors is much greater." It will be noted that these quotations from Ewing are concerning tumorous growths in general.

Williams in conclusion of a chapter on multiple primary carcinoma wrote "Multiple outbreaks of malignant disease are probably less exceptional than is generally believed, the evidence to this effect being especially strong in the case of paired organs," and "taken in their entirety, these various considerations point to some general systemic change, as the predisposing cause of primary multiplicity."

Woolley's expressed views in this respect are "Whether the active proliferation is initiated by one or another factor, this would seem evident, that the factor to be effective, the cells must be in a special state, so that whatever acts upon one organ, may affect similarly its pair."

Fried stated that two or more malignant growths occurring at the same time are rare, being found only once in a thousand postmortem examinations. He advanced two reasons for this rarity, and for the average greater age of persons dying with two or more primary malignant neoplasms. (1) A state of immunity is conferred by the first malignant tumor. This belief is based on the experimental work of Jensen and Ehrlich which tended to show that a transplanted malignant growth in mice, confers immunity against a second inoculation of a malignant growth. Later workers, however, have shown that this immunity diminishes after a period. (2) A person with a malignant growth possibly succumbs to his disease before another malignant neoplasm develops. Many workers hold that neoplasms of senescent persons are less malignant than those of younger persons, and it is obvious that persons with mildly malignant neoplasms will live longer and have more chance of a second malignant tumor developing. The latter reason possibly accounts for the fact that most observers believe that persons with multiple primary malignant neoplasms are of more advanced age than persons having only one malignant newgrowth.

Muller found 1,121 subjects with carcinomas at 5,012 postmortem examinations of subjects who were more than twenty years of age. Of these 1,121 subjects, 18 had more than one carcinoma.

The literature on this subject, and the reports of cases, are confusing because there is no agreement as to classifications of malignant growths in general, or as to what constitutes a condition of multiple primary malignant neoplasm.

Bilroth's postulates, which were used as criteria by some of the earlier writers, would exclude a large percentage of the type of cases which have been used by later workers. Most of the statistics found in reports in the literature are based on material obtained at necropsy, and it is evident that such reports usually will include only those cases in which there were two or more primary malignant tumors at the time of death. Studies based on a series of postmortem examinations would not take into account the fact that the person dying of one tumor might have been cured of a previous separate and distinct primary malignant newgrowth. It is also conceivable that many small lesions, such as early epitheliomas of the skin, or some small growth in the organs usually not examined postmortem, would escape attention when the internal organs were the center of the examiner's interest.

Few of the reports in the literature include cases in which there were malignant tumors in paired organs or in the same organ, because of the difficulty of excluding the possibility that the second lesion was metastatic. It is known that cysts of the ovary are often bilateral, and it is also known that certain types of these cysts often become malignant, so it would seem that in cases of bilateral papillary carcinoma of the ovaries the evidence is in favor of two or more primary growths rather than of metastasis from one ovarian tumor to the other ovary. It is agreed by most authorities that polyps of the intestinal canal are often the forerunners of malignant tumors. In cases of multiple polyps, in which more than one malignant growth is found, the conclusion would be that two separate malignant growths had developed on two separate polyps. It is hard

to conceive of multiple basal cell epitheliomas, or widely separated squamous cell epitheliomas of low grade being produced from a single focus. The question concerning simultaneous carcinoma in both breasts, or of carcinoma in one breast, subsequent to that in the other, is not so easily settled. Harrington, in a series of 3,038 cases of carcinoma of the breast, found twenty in which the condition was bilateral. He considered the growths to have been primary in each breast. In 42 of the remaining cases, carcinoma developed in the opposite breast subsequently. In most cases carcinoma of the second breast is considered primary by Harrington, except in cases in which the lesion is obviously metastatic.

Kilgore, in studying 1,100 unselected cases of carcinoma of the breast found the second breast, in 37 instances, to be the site of carcinoma, either simultaneously or subsequently. In 13 of these cases, he considered the lesions were definitely primary, and basing his estimate on statistics of the Census Bureau, he concluded that a woman who has had one breast removed for carcinoma, and survives five years after operation, is about four times more likely to have carcinoma in the remaining breast than is the average woman of her age to have carcinoma of either of her breasts.

Owen, who was cited by Hanlon, has reviewed a large number of cases of malignancy seen at the Barnard Free Skin and Cancer Hospital, multiple malignant tumors were found in 4.7 per cent of 3,000 cases. Although the present study is somewhat similar to Owen's, the material used is different. His cases were from a clientele which presents a relatively large percentage of cutaneous carcinomas, and those on which this report is based were from a clientele which presents a large number of patients with carcinoma of the internal organs. Owen also used some cases in which only a clinical diagnosis was made.

#### PRESENT STUDY

As has been said, there were 2,124 patients at The Mayo Clinic in 1929, in whose cases a microscopic diagnosis of malignant neoplasm was made. At the time of this study, 71 of these patients had also had another primary malignant neoplasm or more. This shows the percentage of proved cases of multiple malignant neoplasm in this series to have been 3.34. These cases were reviewed in 1931, or two years after one of the malignant tumors in each case had been observed. There were 15 patients whose first malignant tumor existed more than two years before 1929, that is, in 1926 or before. Therefore, it is reasonable to assume that about 15 of the 2,053 patients with single malignant tumors seen in 1929 will, at some time later than 1931, have another primary malignant tumor. This reasoning would bring the total number of persons with multiple primary malignant tumors, past and future, to a theoretic total of 86, or 4 per cent of the 2,124 cases of malignant neoplasms reviewed.

If this study included, also, those cases in which only clinical diagnosis was made, the percentages of cases of multiple primary malignant neoplasms would have approached or exceeded those of Owen.

There were 152 separate malignant lesions among the 71 patients. In making this count and subsequent counts patients who were reported to have multiple lesions, but in whose cases the number of such lesions was not stated, are counted as having only 2 lesions. Of the 152 lesions, 58 originated in the skin, and were divided as follows: 37 squamous cell epitheliomas (Figs 1 and 2), 11 basal cell epitheliomas, 9 mixed basal cell and squamous cell epitheliomas and one melano epithelioma. There were 31 malignant neoplasms of the breasts, 18 of the ovaries, 17 of the colon, 3 of the parotid glands, and 1 each of the stomach, gall bladder, thyroid gland, and testis, all of which were adenocarcinomas. Of the 10 neoplasms of the uterus, 8 were adenocarcinomas (Figs 3 and 4), 1 a squamous cell epithelioma,

and 1 a sarcoma. There were 4 squamous cell epitheliomas of the mouth, pharynx and larynx, and 4 of the urinary bladder (Figs 5 and 6) and urethra. Metastatic squamous cell epitheliomas of the lymph nodes were found in 2 cases in which the



Fig. 1—(Case 8) Squamous cell epithelioma of the upper jaw. Graded 2 ( $\times 110$ )

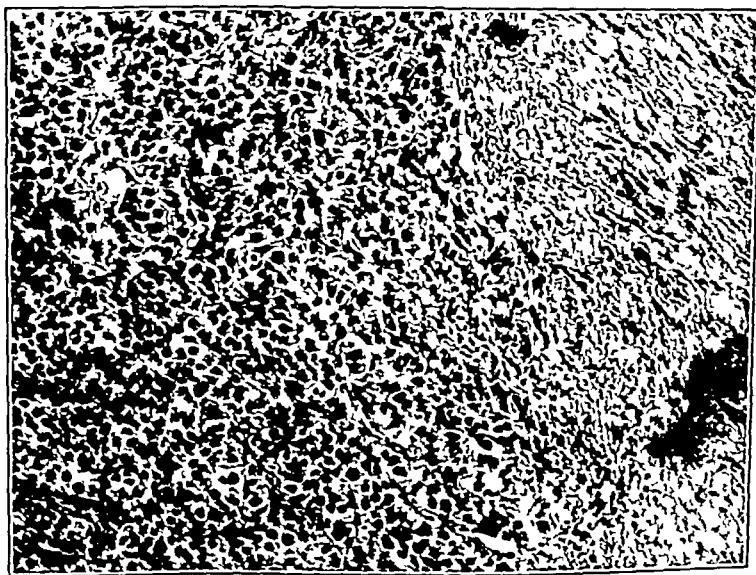


Fig. 2—(Case 8) Squamous cell epithelioma of the lower lip. Graded 4 ( $\times 110$ )

primary lesion was not known. There was one case of lymphosarcoma of the inguinal region.

Table I includes data on each of the 71 cases.



For better study, the cases have been divided into seven groups. Some cases of necessity are included in more than one group.

*Group 1 Squamous Cell Epithelioma of the Skin*—Thirty-two patients had

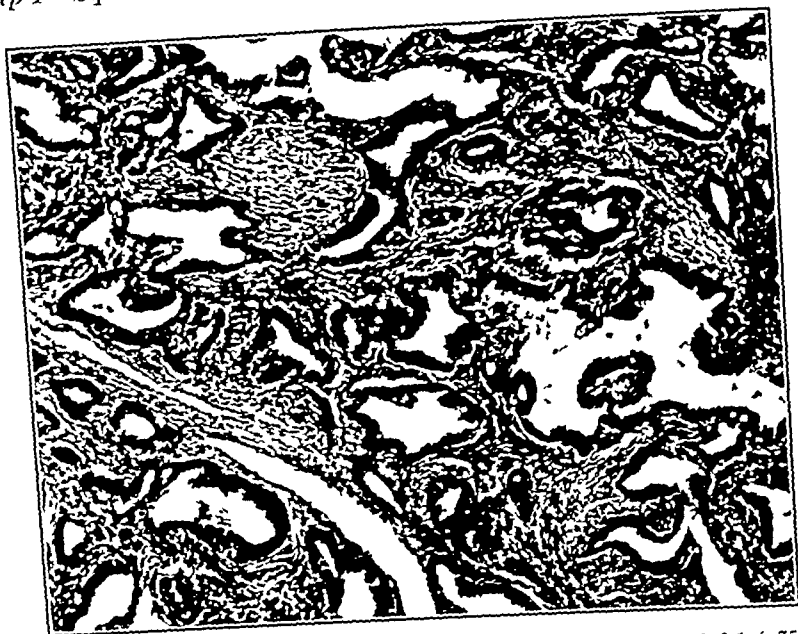


Fig. 3—(Case 61) Adenocarcinoma of the body of the uterus Graded 1 ( $\times 75$ )

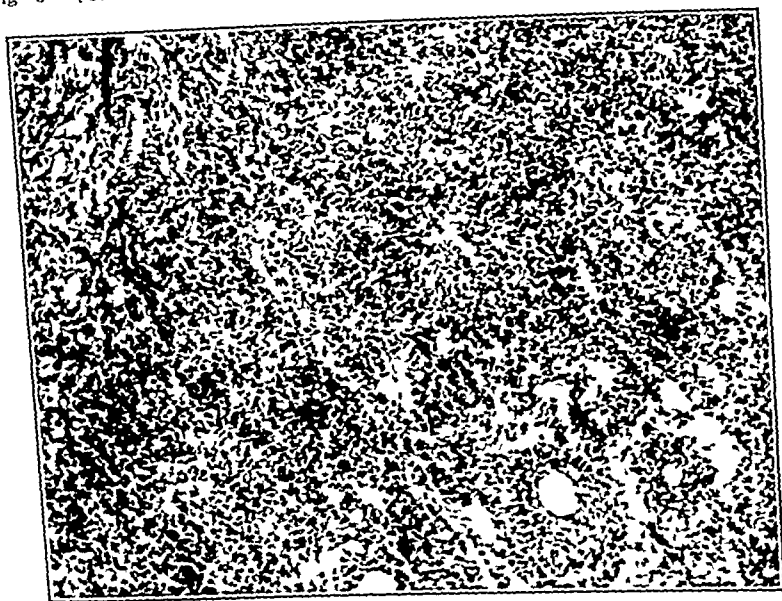


Fig. 4—(Case 61) Adenocarcinoma of the body of the uterus Graded 4 ( $\times 75$ )

one or more squamous cell epithiomas of the skin. In these 32 cases there were 46 squamous cell epithiomas of the skin, 9 of which were superimposed on basal cell epithelioma. There were in this group 27 men and 5 women, 28.1 per cent of whom

gave family histories of malignant neoplasms. The average\* age at which these lesions appeared was fifty-seven and five tenths years. The average\* grade of malignancy for the group was 1.8



Fig 5 — (Case 70) Squamous cell epithelioma of the urinary bladder Graded 1 (x110)

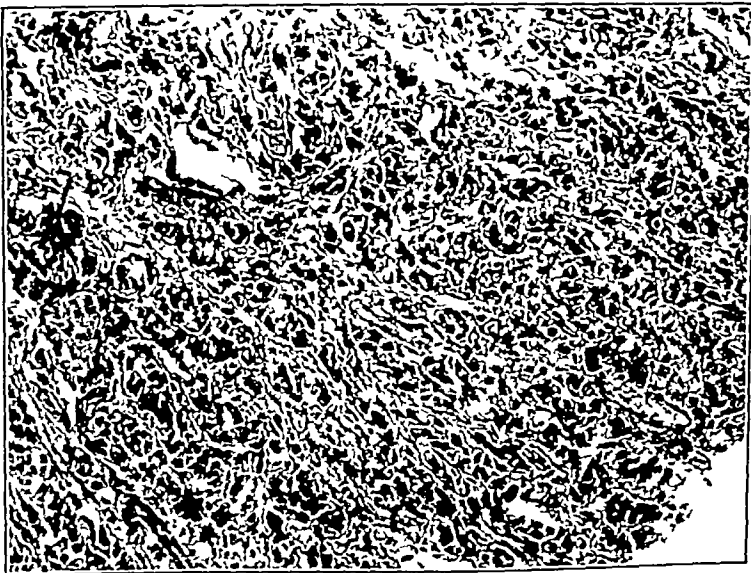


Fig 6 — (Case 70) Squamous cell epithelioma of the urinary bladder Graded 4 (x110)

\*As far as our material is concerned the manner of computing the average in this group and elsewhere in the paper unless the term average is qualified can best be explained by an example. Say that there were two patients in a group. The first growth of one patient was found when he was fifty-two years of age the second growth when he was sixty. The first growth of the other patient was found when he was sixty years of age the second growth when he was sixty eight. The average age for this group would be the average of all the ages just given or sixty. Say that one of these two patients had two growths graded respectively 1 and 3. The other patient had two growths graded respectively 2 and 4. The average grade of malignancy for the group therefore, is 2.5

*Group 2 Basal Cell Epithelioma*—In this group there were sixteen patients, of whom twelve were men and four were women. Of the group 37.5 per cent gave family histories of malignant neoplasm. The average age at which the basal cell epitheliomas appeared was fifty-three and four-tenths years.

*Group 3 Neoplasms of the Large Bowel*—This group comprises 9 patients, 5 men and 4 women. Six of these patients had more than one primary malignant neoplasm of the large bowel, and 5 had malignant neoplasms other than of the bowel. The age given, in most of the cases, is the age of the patient at the onset of symptoms. The average age when symptoms were noticed was forty-four and three tenths years. There was a family history of malignant neoplasms in 44.4 per cent of the cases.

*Group 4 Neoplasms of the Breast*—Carcinoma of the breast afflicted 18 persons. More than one primary malignant neoplasm of the tissue of the breast occurred in 13, one of whom had two different carcinomas in the same breast (Case 39). The lesions of the breast appeared at an average age of forty-seven and five-tenths years. One patient with carcinoma of the breast was a man (Case 31).

The grades of malignancy of the 31 newgrowths in the breast were as follows: Grade 1, six growths (19.4 per cent), Grade 2, two growths (6.4 per cent), Grade 3, eleven growths (35.5 per cent), and Grade 4, twelve growths (38.8 per cent). The average grade of malignancy was 2.9.

Of the 18 persons who had carcinoma of one breast, 12 had carcinoma of the opposite breast, but of the 12 who had bilateral carcinoma of the breast only 3 had demonstrable nodal metastasis. Five years elapsed in one of the patients in whom there was metastasis before the appearance of the growth in the second breast. This leaves only two of the bilateral cases of carcinoma of the breast in which there is any doubt as to whether both tumors were primary.

In Case 47 there was a family history of marked susceptibility to malignant tumors. There was a positive family history in 33.3 per cent of the cases.

*Group 5 Neoplasms of the Ovaries*—There were 7 cases of bilateral malignant tumors of the ovary among the total of 11 women who had malignant tumors of ovarian origin. The average grade of malignancy was 2.3, and in 10 per cent of the cases in which the family history was recorded, other members of the family had malignant tumors.

*Group 6 Neoplasms of the Uterus*—In 7 of the cases of malignant newgrowths of the female genitalia, the newgrowths were in the uterus, all of which were carcinomas except 2. Of these 2, one was a sarcoma of the uterus, and the other, a squamous cell epithelioma of the cervix. One patient had 2 primary malignant tumors in the uterus (Case 9). The average grade of uterine malignant growths was 2.2. Two patients (28.6 per cent) gave histories of malignant neoplasms afflicting other members of their families. In every case in which glandular epithelium of the uterus was involved, the associated lesion was in the breast or genitalia.

*Group 7*—This contains the 3 cases not included in any of the other groups. The tabulated data for this group are shown in Table I, Cases 69, 70, and 71.

#### COMMENT

*Groups 1, 5, and 6*—Attention has been called to the fact that if a malignant neoplasm develops in one organ of a system, another primary malignant neoplasm

TABLE I  
SUMMARY OF CASES OF MULTIPLE PRIMARY MALIGNANT NEOPLASMS

CASE	SEX	OTHER MEMBERS OF FAMILY WHO HAVE MULTIPLE GROWTHS	FIRST GROWTH		GRADE OF MALIGNANCY	AGE OF PATIENT	ASSOCIATED GROWTH OR GROWTHS		GRADE OF MALIGNANCY	AGE OF PATIENT
			SITUATION AND TYPE OF GROWTH	SITUATION AND TYPE OF GROWTH						
1	M	0	Mouth, squamous cell epithelioma	Mouth, squamous cell epithelioma	1	52	Mouth, squamous cell epithelioma		3	52
2	F	1	Nose, squamous cell epithelioma		1	53	Right upper eyelid, squamous cell epithelioma		4	56
3*	M	0	Left first finger, squamous cell epithelioma		1	55	Right second finger, squamous cell epithelioma		1	55
4*	M	0	Finger, squamous cell epithelioma		1	52	Finger, squamous cell epithelioma		1	53
5	M	0	Right side of face, squamous cell epithelioma		1	70	Left side of face, squamous cell epithelioma		1	70
6	F	1	Left side of neck, squamous cell epithelioma		1	65	Left outer canthus, squamous cell epithelioma		1	69
7	M	1	Right cheek, squamous cell epithelioma		1	52	Left side of lower lip, squamous cell epithelioma		1	54
8	M	0	Upper jaw, squamous cell epithelioma		1	60	Lower lip, squamous cell epithelioma		3	60
9	M	0	Face, basal and squamous cell epithelioma		1	75	Face, basal and squamous cell epithelioma		4	75
10	M	0	Face, squamous cell epithelioma		1	46	Face, basal and squamous cell epithelioma		1	46
11	F	1	Face, basal and squamous cell epithelioma		1	30	Nose, basal and squamous cell epithelioma		1	33
12	M	0	Right inner canthus, basal cell epithelioma		1	25	Left cheek, squamous cell epithelioma		1	55
13	M	1	Lower lip, squamous cell epithelioma		1	75	Left cheek, squamous cell epithelioma		3	76
14	F	3	Left side of face, basal and squamous cell epithelioma		1		Upper lip, basal and squamous cell epithelioma		1	76
15	M	0	Nose, basal and squamous cell epithelioma		1	20	Lip, basal cell epithelioma		1	43
16	M	0	Left preauricular region, basal cell epithelioma		2	59	Prepuce, squamous cell epithelioma		1	62
17	M	0	Left cheek, basal cell epithelioma		1	61	Lower lip, squamous cell epithelioma		1	65
18	M	1	Right and left regions, squamous cell epithelioma		1	67	Lower lip, squamous cell epithelioma		2	77
19	M	0	Lower lip, squamous cell epithelioma		1	64	Back, basal cell epithelioma		1	64
20	M	0	Upper lip, basal and squamous cell epithelioma		1	13	Left cheek, basal cell epithelioma		1	43
21	F	0	Right parotid gland, adenocarcinoma		1	48	Right parotid gland, adenocarcinoma		1	51
					1	42	Right heel, squamous cell epithelioma		1	36

\*Workers with ionizing rays  
\*\*Inoperable carcinoma of stomach at age of forty two years  
†Also an adenocarcinoma of tonsil in uterus

TABLE I (CONTINUED)

TABLE I (Continued)										
CASE	SEX	OTHER MEMBERS OF FAMILY WHO HAVE MALIGNANT GROWTHS	FIRST GROWTH		GRADE OF MALIGNANCY	AGE OF PATIENT	ASSOCIATED GROWTH OR GROWTHS		GRADE OF MALIGNANCY	AGE OF PATIENT
			SITUATION AND TYPE OF GROWTH	SITUATION AND TYPE OF GROWTH						
22	M	0	Lower lip, squamous cell epithelioma	Left testis, adenocarcinoma	1	33	Nasopharynx, squamous cell epithelioma	1	33	
23	M	0	Lower lip, squamous cell epithelioma	Lower lip, squamous cell epithelioma	1	67	Lower lip, squamous cell epithelioma	1	68	
24	M	0	Inside cheek, squamous cell epithelioma	Lower lip, squamous cell epithelioma	1	45	Left mandibular region, squamous cell epithelioma	1	46	
25	M	0	Mouth, squamous cell epithelioma	Inside cheek, squamous cell epithelioma	1	64	Rectum, adenocarcinoma	1	65	
26	M	1	Lower lip, squamous cell epithelioma	Lower lip, squamous cell epithelioma	1	75	Lower lip, squamous cell epithelioma	1	78	
27	M	0	Transverse colon, adenocarcinoma	Transverse colon, adenocarcinoma	1	51	Lower lip, squamous cell epithelioma	1	60	
28	M	0	Transverse colon, adenocarcinoma	Transverse colon, adenocarcinoma	3	64	Nose, basal and squamous cell epithelioma	1	55	
29	M	0	Larynx, squamous cell epithelioma	Larynx, squamous cell epithelioma	2	63	Lower lip, squamous cell epithelioma	1	63	
30	M	0	Right parotid gland, adenocarcinoma	Right parotid gland, adenocarcinoma	1	46	Lower lip, squamous cell epithelioma	1	65	
31	M	0	Glands of neck, squamous cell epithelioma	Right parotid gland, adenocarcinoma	3	54	Nose, squamous cell epithelioma	1	68	
32	M	1	Lower lip, squamous cell epithelioma	Inner cheek, squamous cell epithelioma	2	68	Inner cheek, squamous cell epithelioma	3	81	
33	M	0	Stomach, adenocarcinoma	Breast, adenocarcinoma	3	61	Breast, adenocarcinoma	1	66	
34	M	1	Pancreas, basal cell epithelioma	Nose, squamous cell epithelioma	1	?	Nose, squamous cell epithelioma	1	?	
35	M	2	Nose, basal cell epithelioma	Pancreas, basal cell epithelioma	1	33	Pancreas, multiple lesions, basal cell epithelioma	1	52	
36	F	0	Left cheek, basal cell epithelioma	Right breast, adenocarcinoma	1	60	Right breast, adenocarcinoma	4	62	
37	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	34	Left breast, adenocarcinoma	1	63	
38	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	59	Right breast, adenocarcinoma	3	78	
39	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	40	Stomach, adenocarcinoma	1	61	
40	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	2	74	Right breast, adenocarcinoma	1	47	
41	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	3	59	Ovary, adenocarcinoma	2	47	
42	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	3	40	Same breast, adenocarcinoma	1	43	
43	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	4	43	Right breast, adenocarcinoma	1	43	
44	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	2	42	Right breast, adenocarcinoma	1	52	
45	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	3	51	Right breast, adenocarcinoma	3	37	
46	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	3	36	Left breast, adenocarcinoma	3	39	
47	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	38	Right breast, adenocarcinoma	1	42	
48	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	41	Right breast, adenocarcinoma	1	42	
49	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	41	Right breast, adenocarcinoma	1	42	

TABLE I (CONTINUED)

CASE	SEX	OTHER MEMBERS OF FAMILY WHO HAVE MALIGNANT GROWTHS	FIRST GROWTH		GRADE OF MALIGNANCY	AGE OF PATIENT	ASSOCIATED GROWTH OR GROWTHS		GRADE OF MALIGNANCY	AGE OF PATIENT
			SITUATION AND TYPE OF GROWTH	SITUATION AND TYPE OF GROWTH						
45	F	1	Left breast, adenocarcinoma	Left breast, adenocarcinoma	1	42	Right breast, adenocarcinoma		4	44
46	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	4	56	Right breast, adenocarcinoma		4	62
47**	F	4	Right breast, adenocarcinoma	Right breast, adenocarcinoma	3	34	Left breast, adenocarcinoma		3	35
48	F	0	Right breast, adenocarcinoma	Right breast, adenocarcinoma	3	45	Left breast, adenocarcinoma		3	50
49	F	0	Left breast, adenocarcinoma	Left breast, adenocarcinoma	4	55	Right breast, adenocarcinoma		4	56
50	F	0	Scal right breast, adenocarcinoma	Scal right breast, adenocarcinoma	3	44	Left breast, adenocarcinoma		4	46
51	F	No data	One ovary, papillary adenocarcinoma	One ovary, papillary adenocarcinoma	2	39	Other ovary, papillary adenocarcinoma		2	39
52	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	3	66	Other ovary, adenocarcinoma		3	66
53	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	1	33	Other ovary, adenocarcinoma		1	33
54	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	4	52	Other ovary, adenocarcinoma		4	52
55	F	0	One ovary, papillary adenocarcinoma	One ovary, papillary adenocarcinoma	2	35	Other ovary, papillary adenocarcinoma		2	35
56	F	1	One ovary, papillary adenocarcinoma	One ovary, papillary adenocarcinoma	1	44	Other ovary, papillary adenocarcinoma		1	44
57	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	3	44	Other ovary, adenocarcinoma		3	44
58	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	4	60	Uterus, papillary adenocarcinoma		1	60
59†	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	2	46	Uterus, papillary adenocarcinoma		2	46
60	F	0	One ovary, adenocarcinoma	One ovary, adenocarcinoma	1	51	Uterus, adenocarcinoma		1	51
61	F	1	Uterus, adenocarcinoma	Uterus, adenocarcinoma	4	38	Uterine polyp, adenocarcinoma		1	51
62	F	1	Rectum, adenocarcinoma	Rectum, adenocarcinoma	2	17	Uterine polyp, adenocarcinoma		1	38
63	F	0	Colon, 3 growths, adenocarcinoma	Colon, 3 growths, adenocarcinoma	1 and 2	37 to 45	Uterus, squamous cell epithelioma		2	48
64	M	0	Rectum, adenocarcinoma	Rectum, adenocarcinoma	2	50	Uterus, sarcoma		4	45
65	F	0	Rectosigmoid, adenocarcinoma	Rectosigmoid, adenocarcinoma	3	50	Multiple rectal polyp, adenocarcinoma		3	50
66	F	1	Rectosigmoid, adenocarcinoma	Rectosigmoid, adenocarcinoma	2	50	Rectal polyp, adenocarcinoma		3	50
67	M	0	Rectum, adenocarcinoma	Rectum, adenocarcinoma	2	24	Rectal polyp, adenocarcinoma		1	24
68	M	0	Rectum, adenocarcinoma	Rectum, adenocarcinoma	1	42	Rectum, adenocarcinoma		2	42
69	F	0	Urinary bladder, squamous cell epithelioma	Urinary bladder, squamous cell epithelioma	2	53	Urethra, squamous cell epithelioma		2	42
70	M	0	Urinary bladder, squamous cell epithelioma	Urinary bladder, squamous cell epithelioma	1	68	Gall bladder, adenocarcinoma		4	54
71	F	0	Thyroid, adenocarcinoma	Thyroid, adenocarcinoma	1	58	Urinary bladder, squamous cell epithelioma		1	59
					2	29	Left groin, lymphosarcoma		2	32

is more likely to develop in that organ or in an organ of the same system. In the 29 patients with malignant neoplasms derived from the glandular epithelium of the organs peculiar to the female sex, there were only three proved malignant neoplasms in organs that are common to both sexes. A patient who had had carcinoma of both breasts was found to have an inoperable carcinoma of the stomach at operation, but this patient is not included, because no tissue for microscopic diagnosis of the lesion in the stomach was obtained (Case 47).

*The Grade of Malignancy*—The relationship of the occurrence of multiple malignant tumors to the degree of malignancy of these tumors is one of the objects of this study.

Since one of us (Broders) in 1920, published the method of grading the malignancy of squamous cell epithelioma of the lip, it has been extended and accepted in the grading of other types of malignant neoplasms in various situations.

The grade of malignancy has been determined for the 152 lesions, and is recorded in Table I. The average grade of malignancy varies directly with the number of lesions occurring in any one age group, as is shown in Table II. In other

TABLE II  
GRADE OF MALIGNANCY OF THE NEOPLASMS

AGE GROUPS, YEARS	NEOPLASMS					
	TOTAL NUMBER	NUMBER IN DIFFERENT GRADES				AVERAGE GRADE
		1	2	3	4	
20 to 29	6	4	2			1.33
30 to 39	28	11	8	6	3	2.0
40 to 49	34	14	7	7	6	2.14
50 to 59	37	10	12	6	9	2.48
60 to 69	36	11	11	5	9	2.33
70 and above	11	6	3	2	0	1.64
Total	152	56	43	26	27	2.15

words, if a person has one malignant tumor, the probability of the development of another malignant tumor varies directly with the grade of malignancy of the first tumor, presuming that the person survives long enough for another malignant new-growth to develop. This leads to the hypothesis that the unknown factors which cause development of a single malignant neoplasm are exaggerated in cases of multiple malignant neoplasm and express themselves in both the grade of malignancy and the frequency of multiple malignant neoplasms.

If this is true then the occurrence of multiple primary malignant tumors should be more common than would be expected if they were merely accidental. This study, and that of Owen, tend to substantiate the hypothesis that multiple primary malignant tumors actually are of more than accidental occurrence in spite of the fact that the probability of the development of a malignant tumor is not known for any control group. Also if the hypothesis is true, then the average grade of malignancy of multiple primary malignant tumors should be higher than in a like group of single malignant tumors. The proof of this is beyond the scope of this study.

*Hereditary Factors*—There are many opinions concerning the part that hereditary influences may play in the causation of malignant neoplasms of human beings. The family histories in most clinical records are lacking in detail and thoroughness, and are wholly inadequate for any statistical data of great value.

In the present study, there was some record of the family history in 70 cases. In 20 cases (28.6 per cent) there was a history of malignant tumors in other members of the family. Three patients gave histories of more than one member of the family having malignant tumor.

Case 47 is interesting in this respect. The woman had a carcinoma in one breast, and then in the other at the ages, respectively, of thirty-four and thirty-five years. At the age of forty-two years, at abdominal exploration, an inoperable lesion which was taken to be malignant was found in the stomach. A note on her history said that she came of a "cancer family," and this is substantiated by the fact that her mother and three of her sisters had had carcinoma of the breast.

*Sex*—In this series, nothing of interest was found with relation to distribution by sex. Thirty-two of the patients were men and thirty-nine women.

*Age*—Hanlon found the average age of patients with multiple carcinomas, as reported in the literature, to be fifty-eight and eight-tenths years. In his own series of cases in which necropsy was performed, the average age was sixty-two and six-tenths years. Among Owen's cases in which ages were given, the average was sixty-two and one tenth years. Owen, however, did not take into consideration the age at which the lesions appeared.

In the present study, the average age at which the lesions appeared was fifty and four-tenths years. Difficulty was experienced in some cases in estimating the age at which the lesions appeared, and in these cases, as a rule, the age at the onset of symptoms was taken to be the age at which the tumor developed. This is, obviously, an inaccurate method, but the best that could be used.

#### SUMMARY AND CONCLUSIONS

Persons are more likely to have more than one primary malignant neoplasm than a review of the literature would lead one to believe.

Multiple primary malignant neoplasms occur most commonly in the same organ, or in organs of the same system.

Of the patients with malignant neoplasms seen at The Mayo Clinic in one year, 3.4 per cent have had more than one primary malignant neoplasm. Reasons have been presented to show that the percentage is even higher than this.

From the study of cases of multiple malignant neoplasms, it seems that the factors which cause the development of the tumors also express themselves in the grade of malignancy of the tumors.

A large percentage of patients with multiple primary malignant neoplasms give family histories which include other members who have had malignant tumors.

No conclusion as to the occurrence of multiple malignant neoplasms, with reference to sex, could be drawn.

In this series, the average age at which the growths developed was fifty and four-tenths years.

Seventy-one cases of multiple primary malignant neoplasms have been added to the literature on this subject.



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## STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION\*

## IV FACTORS OF CARBOHYDRATE METABOLISM IN MUSCLE REPAIR

D K. FISHBACK, PH D , AND H R FISHBACK, SC D , M D , CHICAGO, ILL

IN A previous article,<sup>1</sup> we have directed attention to the variations from normal of certain chemical factors in the metabolism of abnormal muscles. In that study a reproducible type of injury of rabbit muscle, with a standard method of trauma, was used.<sup>2</sup> The acute molecular degeneration of muscle resulting was studied in its florid state.

Briefly, the method of injury consisted in contusing the muscle while the animal was under light anesthesia. With the leg extended on a well padded wooden block the gastrocnemius muscle was struck a number of scattered light blows with a light, rubber covered iron rod. In this manner the injury produced is diffuse, and the depth of trauma can be gauged very well at its inception. The gastrocnemius is well enough isolated that it can be removed easily for study.

A series of animals with such muscle injury was carried through the stage of repair. Complete study was made of the gross and microscopic changes occurring in the muscle during the process of repair.<sup>2</sup>

Associated with this morphologic study, chemical analyses of the muscles were performed for the entire repair series, with a view to correlating the course of carbohydrate metabolism in the stage of development of the injury, with that found during the break-up and removal of the injured muscle and its replacement by new growing muscle cells.

*Methods and Results*—The methods used have been presented in the above-

\*From the Department of Pathology, Northwestern University Medical School.  
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given references. Results in series were obtained for glycogen, lactic acid, and organic and inorganic phosphorus. These are presented in the accompanying figure.

#### DISCUSSION

Studies of glycogen distribution in tissues are often made by staining methods with the purpose of obtaining relative proportions of the glycogen present. Gly-

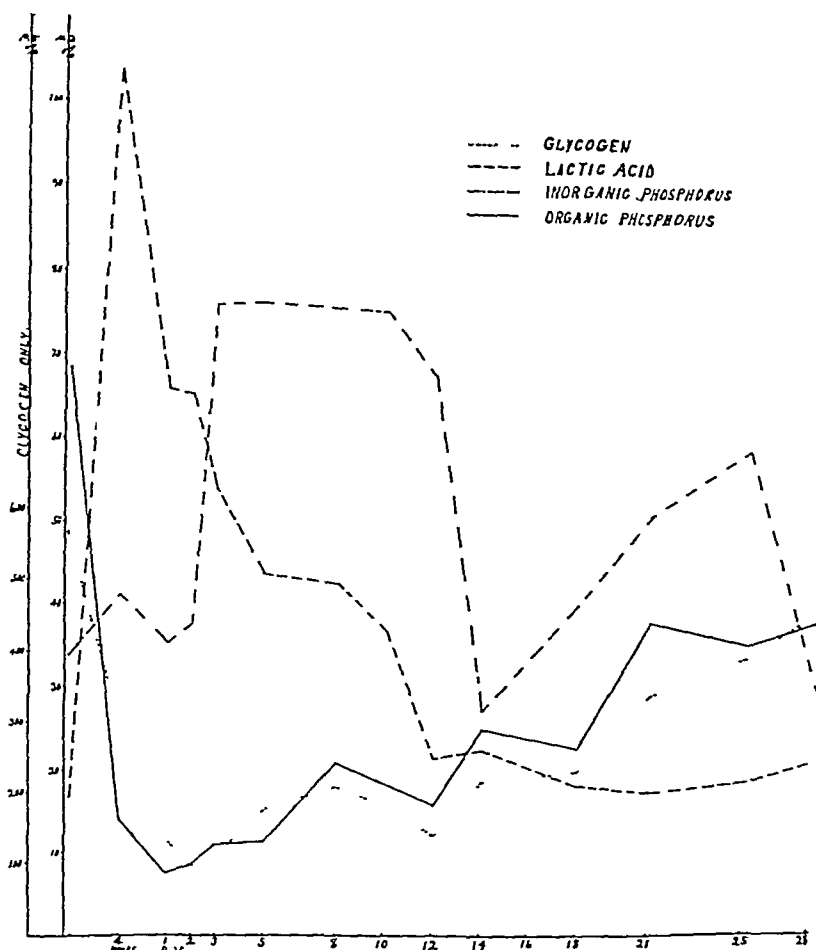


Fig 1—Carbohydrate metabolism during muscle repair

cogen in the tissues, however, is quite labile. Such factors as the length of time elapsing from the death of the animal, or removal of the tissue, to the time the tissue is fixed, the size of the tissue block to be fixed, the amount of handling or trauma of the tissue, and many other things may vary the amount of glycogen present. It is evident that the study of glycogen by the staining method cannot compare in accuracy or informative value with quantitative chemical recovery of glycogen.

One phase of this question was studied by Naka<sup>3</sup> on brain tissue. It was found that glycogen in stained preparations became visible as granules at a concentration

of about 0.01 per cent when the magnification was 500 times, and that a concentration of about 0.03 per cent was necessary to see granules at a magnification of 50

In the given figure glycogen decreases quite rapidly to a low point of 9.4 mg per cent at the end of forty-eight hours. From that point there is a gradual rise to nearly the control value at the end of the observation period. From microscopic study of repair of the muscles<sup>2</sup> the glycogen increase in repair is found to be synchronous with the new growth of muscle cells.

Craigm<sup>4</sup> found that the glycogen content of regenerating muscle was lower than normal but varied considerably in different parts of the tissue. His conclusions are based on staining of glycogen in histologic sections, which, as previously stated, is open to criticism in regard to either absolute or relative values.

It might be expected that rapidly growing muscle cells would contain much glycogen for use in growth metabolism. In embryonic muscle tissue glycogen has been stated to appear early, and to accumulate in rich amounts.<sup>5, 6, 7</sup> However, it would appear from our studies that in repair of this type of injury in muscles, there is no marked storage of glycogen as a reserve supply. It is formed, instead, in amounts somewhat proportionate to those found in normal muscle.

Lactic acid shows a very rapid rise to a high point of 103.3 mg per cent in four hours, and a rather rapid decrease thereafter. Its formation might be expected to run parallel to the decrease of glycogen but, as is seen, the lowest glycogen is reached only after forty-eight hours.

The early accumulation of lactic acid, then, is not accounted for by the amount of glycogen used up. A similar discrepancy under different experimental conditions was noted by Olmsted and Coulthard<sup>8</sup> in their work on fatigued muscles and by Ronzoni<sup>9</sup> in muscle hash. It is possible that the early excess lactic acid might arise from lactacidogen if sufficient quantities of that substance were present.<sup>10</sup> It is evident that it cannot have had origin from hexose phosphate because of the relatively insignificant increase of inorganic phosphorus at the time of highest lactic acid value.

The later fall of lactic acid, while glycogen continues to be broken up, may be accounted for by the high diffusibility of lactic acid, so that it is rapidly taken up by the circulating blood. It is not likely that the degenerated muscle cells function at a higher level to oxidize increased amounts of lactic acid. As repair progresses lactic acid is seen to decrease gradually down to almost control value.

The phosphocreatine curve runs somewhat parallel to that of glycogen, with a sharp drop during the twenty-four and forty-eight hour periods, and a later gradual rise during the period of repair, although the final value of both is considerably under that of the control. There is, however, no direct molar relationship in the variations of the two substances.

Also the phosphocreatine decrease is not proportional to the rise of lactic acid in the stage of acute injury although there is a general tendency toward this inverse relationship. Similar lack of proportion was found by Olmsted, Macklei, and Simpson<sup>11</sup> who state that in stimulated frog gastrocnemii there is much greater accumulation of lactic acid than can be neutralized by the amount of phosphocreatine hydrolyzed.

Pickelhirring and Van Hoogenhuyze<sup>12</sup> found an increase of creatine in the

muscles in rigor, and in increased tonus produced in Sherrington's decerebrate rigidity, which might be accounted for now by an increased hydrolysis of phosphocreatine in the muscles. Creatine has for many years been associated in the literature with muscle function, and generally considered to have to do with tonic functions of muscle. For example, Pekelharing<sup>13</sup> found an increased creatinine excretion in men with increased tonicity of muscles caused by long standing erect at attention, or by long continued electrical stimulation, without contraction. Exercise for the same period of time did not give increase of creatinine excretion.

Inorganic phosphorus likewise shows a tendency toward an inverse relationship to phosphocreatine, in that it is generally higher than the control value during the period of repair and sinks toward the end of that period. In active and resting muscles variability of inorganic phosphate may be due to the breakdown and reformation of hexose phosphate. Irving and Bastedo<sup>14</sup> found variable amounts of inorganic phosphorus formed in exercised muscle in relation to lactic acid and phosphocreatine.

#### SUMMARY

In acute molecular degeneration of striated muscle in rabbits, there is a sudden rise of lactic acid which reaches a high point in four hours. Thereafter it declines, rapidly at first, and later more gradually, to about normal value toward the end of the observation period of twenty eight days.

Glycogen drops to a low point within forty-eight hours, but its decrease shows no close ratio to the amount of lactic acid formed. The later increase in glycogen during repair brings it very close to the control range.

Phosphocreatine decreases rapidly, while inorganic phosphorus increases somewhat late and irregularly. This inverse relationship persists through the repair stage, with a slowly rising phosphocreatine value, and a very irregular inorganic phosphorus which tends toward a decreased level.

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## STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION\*

### V NITROGEN METABOLISM OF THE DEGENERATED MUSCLE IN ACUTE INJURY AND REPAIR

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THE skeletal muscles make up the largest mass of protein in the animal body. Many nitrogenous substances are required by them, and yet an understanding of the use of these substances in muscle metabolism has been most difficult to work out. It is assumed that muscle cytoplasm is built up from the amino acids of the blood, and the nucleo-protein partly from blood purines. Creatine has long been associated with muscle tone<sup>1, 2</sup> although its exact relationship to this phenomenon has not been understood. The full rôle of creatine in muscle economy is perhaps not yet completely known, although its important association with carbohydrate metabolism through its combination with phosphoric acid has been presented, by Fiske and Subbarow<sup>3</sup> and Eggleton and Eggleton<sup>4</sup>. Urea was formerly thought to be formed in part in the muscles, until the demonstration by Bollman, Mann, and Magath<sup>5</sup> that this function is performed by the liver only. An important chapter, added by Embden<sup>6</sup> and Parnas<sup>7</sup> is the formation of ammonia in muscle by the deamination of adenine nucleotide, with the formation of inosinic acid. Several other nitrogenous substances are grouped as muscle extractives, without having as yet any ascribed function or, in some cases, without being properly vouched for as constituents of living muscle.

But little attention has been paid, as yet, to the metabolism of nitrogen compounds in abnormal muscle in the animal. In fatigued and in ischemic muscles the study of creatine in relation to phosphates and carbohydrates proved a marked drop in creatin-phosphoric-acid<sup>8, 9</sup>. Muscle ammonia is increased in amount on removal of the muscle from the body, and Parnas<sup>10</sup> states that, in the animal muscle, ammonia is increased in those conditions, such as asphyxia, fatigue, and rigor, which lead to increased lactic acid formation. Such ammonia increase arises, as in normal metabolism, from deamination of adenine nucleotide. The decrease of adenine is practically balanced by an increase of hypoxanthine<sup>7</sup>. The inosinic acid formed is further decomposed by the splitting off of phosphoric acid.

No report has been found in the literature on the nitrogen changes in healing injury of muscles.

*Experimental*—In the present work, chemical studies were carried out on rabbit muscles injured and prepared for analysis according to a procedure pre-

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viously described.<sup>11</sup> The animals were sacrificed at varying lengths of time after injury, up to twenty-eight days, in order to study the changes associated with repair of the injury. Microscopic studies were made on all the muscles removed and are reported in another place.<sup>12</sup> Total nitrogen determinations were made by the Kjeldahl method on 1 gm samples or larger. In the trichloroacetic acid filtrate total nonprotein nitrogen and urea nitrogen were determined according to Folin and

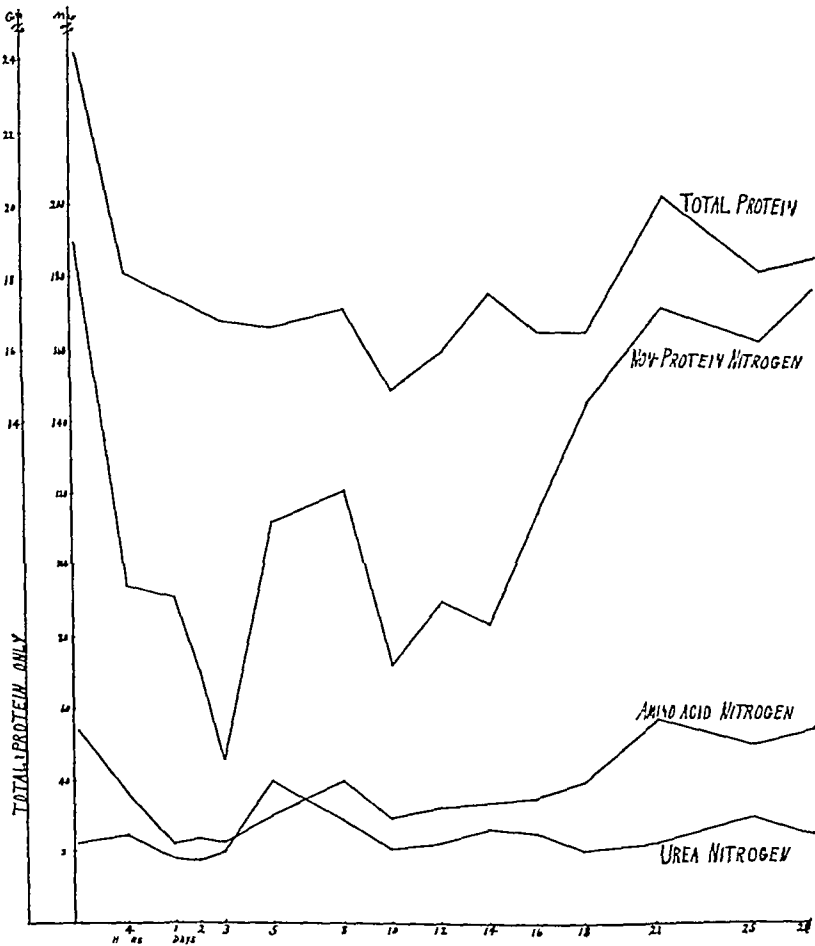


Fig 1—Nitrogen metabolism during muscle repair

Wu,<sup>13</sup> amino acid nitrogen by Folin's method,<sup>14</sup> and phosphocreatine by the method of Fiske and Subbarow.<sup>8</sup> All determinations were made in duplicate.

*Results*—Results are presented graphically in Fig 1.

#### DISCUSSION

The total nitrogen values have been converted to protein by multiplication with the factor 6.25. The protein content of the muscle is found to be decreased during the time of the period of healing. It is probable that the early fall in protein content is influenced to some extent by edema. The digestion of the proteins

of dead muscle cells either by autolysis or by ferments of inwandering cells might also alter the protein mass. However, there is no evidence of such digestion in either the nonprotein or amino acid nitrogen values, since both of these run considerably below control values during the stage of acute muscle injury. On microscopic study of the injured muscles the greater number of exudative cells are found to be mononuclears, many of which are phagocytic.<sup>11</sup> The removal of protein by phagocytosis would give no chemical evidence of the process except in decrease of the total protein value.

The amino acids of the muscles, according to Van Slyke and Meyer,<sup>12</sup> may serve as a reserve, either for energy supply or tissue building. Also they may be intermediate products of tissue building or of tissue break-down. Their origin in the muscle might be from either the ingested food or muscle protein decomposition. Since in our work the amino acids are markedly decreased in the stage of acute injury, and rise slowly only toward the end of the period of observation, there is no indication of break-up of the muscle protein into its component chemical units. If amino acids do serve as a reserve for energy or tissue building, it is likely that sufficient amounts are continually being brought in by the blood, so that there is no need for any marked storage in the tissues. No increase over normal was observed during the stage of active cell proliferation, when an excess of amino acids might have been expected to be on hand for use as the building-stones of new protein molecules. Collip<sup>16</sup> suggested a further possible function for amino acids in maintaining osmotic pressure in the cell, and especially in the nucleus, which has no inorganic electrolytes.

The value for urea nitrogen in the injured muscles fluctuates somewhat throughout the entire observed healing stage from the value in the control animals. The values given for urea nitrogen include also ammonia nitrogen, which in normal muscles is so small in amount in comparison with the urea nitrogen value as to be negligible.<sup>10</sup> In a condition such as heat rigor, however, there is an increase of ammonia nitrogen from a control of about 0.5 mg per cent up to about 7.3 mg per cent.<sup>17</sup> This increased ammonia nitrogen is equal to about 20 to 30 per cent of the corresponding urea nitrogen. With such possibility of ammonia formation in muscle injury, it is likely that our urea nitrogen figures as given are too high by the amount of possible ammonia nitrogen increase. Since the decisive work of Mann, Bollman and Magath,<sup>18</sup> the liver has been accepted as the sole seat of urea formation in the animal. A few workers have been unable to demonstrate urea formation in muscle digests.<sup>18, 19</sup> The lack of constant increase of urea nitrogen in our experiments indicates that in this type of acute molecular degeneration, injured muscles do not have the power *in vivo* of forming urea. The holding power of the injured muscle for urea is likewise but little altered.

Creatine has been determined not as a separate value but as the phosphoric acid compound which is important in carbohydrate metabolism, and is considered in a paper on that subject.<sup>20</sup> It was found to be markedly decreased in amount quite early after the injury with a gradual rise during the period of repair.

The nonprotein nitrogen might be expected to vary in the same muscle with different protein precipitants which release somewhat different amounts of soluble nitrogen. Trichloroacetic acid in 5 per cent solution, which was used uniformly

in our experiments, precipitates proteins but allows a maximum of amino acids and intermediate protein split products to remain in the filtrate<sup>21</sup> It thus extracts protein products of lower grade and likewise other nitrogenous extractives of the muscle not bound to protein in acid solution The total value of such muscle extractives in control animals averages 187 mg per cent of nitrogen In the acute injury stage, the value drops rather rapidly to a low point of 45 mg per cent in three days The recovery is likewise rather rapid, although the value fluctuates considerably, reaching almost to the control level toward the end of the observation period

It is rather striking that these values indicate no autolysis of the degenerated muscles Some factor associated with the vitality of the tissue appears to be active in protecting the degenerated muscles in vivo against the action of autolytic ferments

Microscopically the rapid growth of cells for repair matches the rapid rise toward normal of nonprotein nitrogen in the early stage of repair<sup>11</sup> The supply of nitrogenous food brought by the blood thus appears to be ample to support rapid and continuous formation of the cytoplasm and nuclei of new cells, although apparently no extra supply above the normal is needed

#### SUMMARY

In acute molecular degeneration of striated muscle in rabbits, the decrease of total nitrogen is roughly proportional to the edema, the value rising slowly with subsidence of the edema

The nonprotein nitrogen value falls sharply with the acute injury and rises rather abruptly in the early stage of repair, reaching almost to the control level toward the end of the observation period of twenty-eight days

Urea nitrogen varies inconstantly throughout the acute injury and stage of repair There is no evidence that urea is formed at all, or that it is held in increased amounts, in such injured muscles in vivo

The amino acid value of the degenerated muscle is reduced markedly but rises during the stage of repair to the normal range

There is no evidence presented of autolytic break-down of the muscle mass

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## A COMPARISON OF THE EFFECTS OF MORPHINE, PANTOPON, CODEINE, NARCOTINE, AND PAPAVERINE ON THE RESPIRATION OF RATS AND RABBITS\*

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**I**N SPITE of the rather general agreement among early observers as to the effects of the individual opium alkaloids on respiration, the literature on the pharmacology of various combinations of the alkaloids as compared with morphine is quite contradictory, especially in so far as the toxic, narcotic, and analgetic properties are concerned. The state of our knowledge in this respect is reflected by the statement of Leake (1930), that "little rational evidence exists which would indicate that opium or a mixture of its alkaloids, free from nonalkaloidal material, has any advantage over morphine."

In order to determine the efficiency of the several agents as to their sedative or tranquilizing action as well as for preanesthetic medication, it was deemed necessary to repeat certain of the older studies of the effects of morphine and related compounds on respiration for purposes of correlation with the above observations. The effects of the more important opium alkaloids and pantopon on the respiration of the rat and rabbit are reported in the present study.

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*Methods* —With the exception of morphine, no data on the effects of the opium derivatives on the respiration of the rat are available, possibly because of the size and peculiarities of this animal. The method described by Barbour and Maurer (1920) for measuring the actual expiratory volume of the rat necessitated limitation of the animal's movements, the use of water valves and the insertion of the head in a mask. Results obtained by us with this procedure were so variable that it was discarded.

The procedure followed throughout this study consisted of tying the animals on their backs on specially adapted boards, attaching a lever by means of a thread to a point on the abdomen just below the xiphoid process and recording the respiratory movements on a moving drum. This restraint places the animals under uniform but decidedly abnormal conditions which render the rats more restless than normal. This restlessness is a decided advantage for studying the effects of sedatives.

The data obtained by this method are entirely satisfactory as to the respiratory rates (checked by means of a stop watch) but the volumes recorded are only indirectly representative of the chest movements and are complicated by occasional struggling of the animals. However, the results were sufficiently constant to permit a rather close comparison of the effects of the several agents on the rate and qualitative values of volume. Graphic records were obtained over three satisfactory ten-minute periods during the alternate inhalation of 100 per cent oxygen, and of 95 per cent oxygen, and 5 per cent carbon dioxide before and again forty-five to sixty minutes after, the drug administration.

All drugs were dissolved in distilled water. The concentration in each case was so adjusted that the volume injected (subcutaneously) was as nearly constant as possible. The HCl salts of morphine, papaverine and pantopon and the  $H_2SO_4$  salts of narcotine and codeine were used. The dosages in each case were computed as the base. The basis of equivalence on which morphine was compared with the other agents tested is that usually accepted clinically, i. e., 1 gram of morphine equals 1.66 grams of pantopon, 2 grams of codeine, or 4 grams of papaverine.

#### RESULTS ON RATS

A series of at least 5 (5 to 15) rats were used for every dosage of each of the drugs tested. The normal respiratory rates per minute and the volume (measured excursion of the recording lever in millimeters) was considered as 100 per cent for purposes of statistical comparison with the reactions after medication. The deviation of the rates and volumes from the control values were then computed as percentages of the respective controls. The tabulated data have been expressed as medians in the several illustrations.

*Morphine* —The respiratory changes were noted following the administration of dosages of morphine ranging from 2.1 to 63 mg per kg ( $\frac{1}{2}$  to 15 per cent of the M. L. D. considered as 0.42 gm per kg) of rat. The rate response with small dosages (2 to 3 mg per kg) varied within a range of 8 per cent, while larger dosages were purely depressant. The median changes noted with all dosages tested are illustrated in Fig. 1, which shows that the degree of depression increases rapidly with dosages up to 15 mg per kg and more gradually as the dosage was further

increased. The depression following the administration of the maximal dosage tested was only 3 per cent greater than that observed with 21 mg per kg although the actual difference in dosage was 300 per cent. The respiratory volume diminished progressively with increasing doses over the range tested. The degree of change was negligible with 2.1 mg, increased very gradually and became maximal (50 per cent) following the administration of the maximal dosage (Fig 1). The minute volume as calculated by multiplying the rate per minute by the measured excursions in millimeters, was depressed by all dosages of morphine tested. The depression was minimal (9 per cent) with 2.1 mg per kg, increased rapidly with moderate, more gradually with larger dosages and became maximal (65 per cent) following the administration of 63 mg per kg.

*Pantopon*—The effects of morphine and pantopon (the mixed opium alkaloids) were compared on the basis of weight, therapeutic equivalence, and the morphine alkaloid content, i e, 1 gram of morphine equals 2 grams of pantopon.

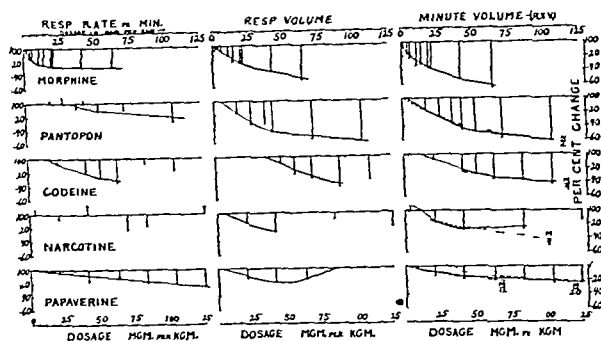


Fig 1—This figure represents the effects of morphine etc. on the respiratory rate, volume and minute volume of the rate. The dosages in milligrams per kilogram of animal are represented on the abscissae. The changes in terms of percentage of the original or normal observations are indicated on the ordinates. Morphine has been superimposed as the broken line (min vol).

The effects of pantopon on the respiration could not be differentiated from those of the morphine content of the dosages administered (Fig 1). The variations in rate after small to medium doses were capricious and smaller in degree than after comparable doses of morphine. However, the differences noted between morphine and pantopon in this respect cannot be explained on the basis of statistical error as volume changes largely compensated for the irregularities in rate, and the calculated minute volumes diminish quite regularly with increasing dosages. This relation is illustrated in Fig 1. Superimposing the curve for the minute volume changes under morphine on that for pantopon by dividing the morphine data by 2, indicates the negligible differences between the two data. The differences are probably within the range of experimental variation. The minor alkaloids of pantopon therefore, do not significantly alter the effects of the morphine contained as concerns respiration. On the basis of therapeutic equivalence, the depressant effects of pantopon were somewhat less than those of morphine but on the basis of morphine equivalence, the two agents were indistinguishable, i e, pantopon (which contains 50 per cent morphine) was almost exactly one half as depressant as in equal weight of morphine.

*Codeine* —The effects of codeine on the respiration were studied with doses ranging from 21 to 105 mg per kg of rat. The depressant effects of small to medium doses were less than one-fourth that of equal weights of morphine. However, with large but subconvulsive doses the degree of depression corresponded very closely to that of a one-fourth dose of morphine (broken line superimposed on codeine minute volume curve). With toxic dosages of codeine (above 105 mg per kg) the depression of rate and volume diminished, presumably by the development of the convulsive action of codeine.

*Narcotine* —The respiratory effects of narcotine are relatively unimportant, although a moderate depression was noted with all dosages tested (Fig 1). *The rate changes* were variable while *the volume* was uniformly depressed. The depression increased gradually to a maximum with 42 mg per kg of rat and either showed little further change or actually diminished. The reactions to small to medium doses were approximately one-fourth those of equivalent doses of morphine, but with larger doses the narcotine depression decreases and as the convulsant action becomes increasingly evident, the depressant effects are overshadowed.

*Papaverine* —The effects of papaverine in the dosages tested were purely depressant. *The rate* diminished progressively with increasing doses. *The volume* also diminished with dosage up to 42 mg per kg but with larger doses the depression largely or completely disappeared. *The changes in minute volume* following the administration of doses up to 60 mg per kg were approximately equivalent to one-twelfth those of similar dosages of morphine. However, in dosages above 75 mg per kg, papaverine was only one-twentieth as depressant as morphine.

#### COMPARISON OF THE STIMULANT EFFECTS OF 5 PER CENT CO<sub>2</sub> ON THE RESPIRATION BEFORE AND AFTER MEDICATION WITH MORPHINE, ETC., WITH RATS

In tabulating the changes in rate, volume, and minute volume following inhalation of 5 per cent CO<sub>2</sub>, the control values noted during the inhalation of oxygen were considered as 100 per cent both before and after medication. The data show the degree of stimulation in percentages of the preceding oxygen control. The legends are self-explanatory.

With drugged rats, the inhalation of 95 per cent oxygen and 5 per cent carbon dioxide following exposure to 100 per cent oxygen, resulted in an increase in rate, volume, and minute volume. The increase in minute volume varied from 80 to 200 per cent above normal with different series of animals with a median increase of 130 per cent for all animals tested. The median increase in rate was 48.5 per cent and that of volume 62.4 per cent. The medians of the entire series are indicated as the normals (marked as X on the ordinates) in all figures. Animals whose normal responses varied by more than 25 per cent from these medians were discarded.

*Morphine* —The stimulant effects of CO<sub>2</sub> on the rate, volume, and minute volume after medication with various doses of morphine are indicated in Fig 2. All doses tested reduced the respiratory response to CO<sub>2</sub>. *The rate reduction* with small to moderate doses was distinctly greater than the accompanying volume changes. With larger dosages, the rate and volume changes were fairly commensurate. The depression of the rate response increased rapidly with small to me-

dium but more gradually with larger dosages. With 21 mg per kg the depression was minimal. The degree of depression with 5 mg was approximately one-third and with the maximal dosage (63 mg per kg) eight-tenths of that observed in the absence of medication. The volume was reduced by all dosages tested, but the marked rate reduction noted with small to moderate doses of morphine was partially compensated for by a relatively smaller effect on the volume. The depression of minute volume increased rapidly with small to moderate and more gradually with larger dosages. After medication with 21 mg per kg (the minimal tranquilizing dose), the degree of stimulation was 42 per cent and after 63 mg per kg only 20 per cent of that noted before medication.

*Pantopon*—The stimulant effects of  $\text{CO}_2$  on the respiratory rate and volume after medication with pantopon (Fig 2) appeared to differ in certain respects from those noted after comparable doses of morphine. Small doses were slightly less and larger doses more depressant to the rate than morphine. The volume response was reduced somewhat with small doses but differed little from the control values

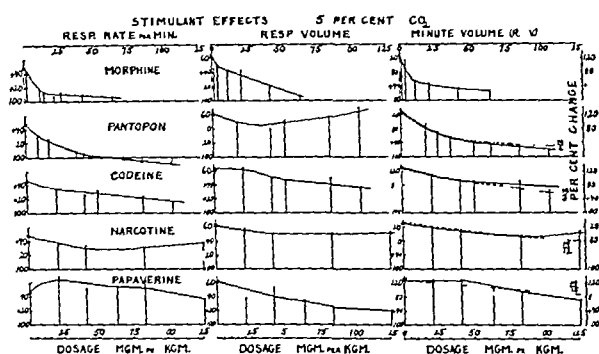


Fig 2—This figure indicates the stimulant effect of 5 per cent  $\text{CO}_2$  after medication with the several agents. The control data after medication was considered as 100 per cent. The curves indicate the change from this medicated level.

after larger dosages in contrast to the reaction noted after morphine. However, the rate and volume changes were compensatory in that the apparent differences between the reactions to pantopon and morphine disappear when the calculated minute volumes are compared, and the curves for pantopon and its morphine content (broken line) are practically identical. It appears probable therefore that the observed differences in rate and volume may have been due to accidental variations in the compensatory adjustment of the animals.

*Codeine*—The stimulant effect of  $\text{CO}_2$  on the respiratory rate was reduced by codeine. The degree of rate change increased gradually with increasing dosage to a maximum of approximately 60 per cent with 105 mg per kg. The volume response was likewise reduced. However with dosages greater than 50 mg per kg little further depression occurred and the median reactions with large doses were actually greater than before medication. Correlation of the stimulant effects of  $\text{CO}_2$  on the minute volume following medication with codeine and morphine (broken line) indicates that small to medium doses of codeine are approximately one-sixth, while larger dosages are distinctly less than one-sixth as depressant as morphine.

*Narcotine*—All dosages tested reduced the normal rate response to  $\text{CO}_2$ . The respiratory volume shows but little in the median figures and the individual experiments exhibit a greater degree of stimulation after than before medication, but the degree of increased response diminished with increasing dosages. The minute volume response to  $\text{CO}_2$  was also invariably greater than normal after narcotine. The stimulant effect of this agent increased to a maximum with a dosage of 42 mg per kg and decreased with larger dosages so that no significant change was evident with a dosage of 126 mg per kg. These data appear to indicate that narcotine in small to moderate doses stimulates the respiratory center. However a depressant action is evident as the degree of stimulation decreases with further increase in dosage.

*Papaverine*—The respiratory rate response to  $\text{CO}_2$  following medication with papaverine in doses up to 105 mg per kg was greater than in the absence of medication. The volume response on the other hand was progressively reduced with increasing dosage. The minute volume, on inhalation of  $\text{CO}_2$ , was distinctly increased above the control values with doses up to 42 mg per kg but further increase in dosage resulted in a progressive depression of the normal response. Papaverine in moderate dosage appeared to stimulate the respiratory center. Large doses (up to 84 mg per kg) are approximately one-fifteenth, while higher dosages are more than one-twelfth as depressant as equivalent weights of morphine.

#### RESULTS ON RABBITS

The procedures followed in the preceding section were repeated on rabbits in order to compare the respiratory responses of the two species to similar experimental conditions as well as to check the results obtained with those reported in the literature. In addition, the actual minute volumes were measured during the inhalation of  $\text{O}_2$  and of a mixture of 95 per cent  $\text{O}_2$  and 5 per cent  $\text{CO}_2$ , both before and after medication with representative doses of the several agents. Measurements of the minute volumes were obtained by tying the animals in a dorsal position, the insertion (under local anesthesia) of a tracheal cannula in order to eliminate the possibility of leakage encountered with the mask method, the use of water valves for control of the air intake and output, and measurement of the expiratory volume by means of a Bohn gas meter. Each 250 cc of the expired air was recorded electrically on a moving drum.

*Morphine*—The respiratory rate was reduced by all dosages tested (Fig. 3). The depressant effects were evident following the administration of the minimal dosage (0.26 mg per kg or 0.1 per cent of the M. L. D.) and increased rapidly with dosage so that the reduction was 80 per cent of the original after medication with 2.6 mg per kg. The degree of depression increased slightly with larger dosages. The volume was reduced by 10 to 25 per cent with dosages between 0.26 and 1.3 mg per kg but was practically unaltered by larger doses in the range tested probably because of a partial compensation for the marked alteration of the rate. The minute volume was actually increased by the minimal dosage tested although the rate and volume changes illustrated in the figures (obtained graphically) indicate a mild depression. With larger doses, the degree of depression of the minute volume increased with the dosage administered.

*Pantopon*—The rate changes following the administration of pantopon differed in no significant respect from those produced by equivalent dosages of morphine. The respiratory volume was diminished by small doses to approximately the same degree as by morphine. With moderate dosages, the volume was increased slightly, thus partially compensating for the distinct accompanying rate depression. After maximal doses, the volume changes were indistinguishable from those of the morphine contained in the preparation. The minute volumes following the administration of small doses of pantopon were more variable than after morphine but the median values were practically the same. Larger doses were somewhat more depressant than morphine but the differences noted probably lie within the range of error and cannot be considered as significant.

*Codeme*—The respiratory rate of the rabbit was depressed by all doses of codeine tested (2.6 to 26 mg per kg). This effect increased with the dosage administered, but the maximal reduction observed was only 20 per cent of the normal or initial readings. Small doses were approximately one-fourth while larger doses

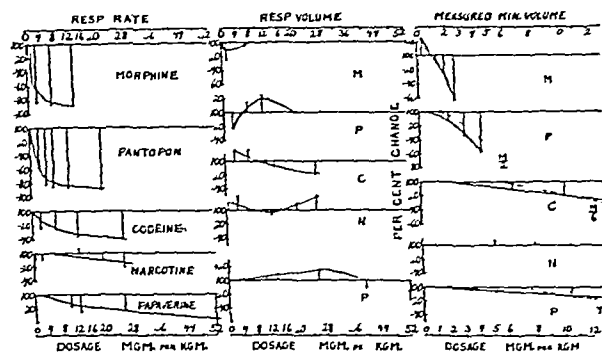


FIG. 3—This figure represents the same data for the rabbit as Fig. 1 for the rat. However the minute volume in Fig. 3 represents the actual gas meter measurements of the changes observed.

were less than one sixth as depressant as equivalent doses of morphine. The volume was increased approximately 15 per cent with dosages below 6.5 mg per kg. Larger doses were depressant but the maximal effect noted was only 15 per cent of the original values. The minute volume changes were insignificant with small to medium dosages although depression was the rule. The reduction observed with the maximum dosage tested was less than one sixth that of equivalent dosages of morphine.

*Narcotine*—The respiratory rate following the administration of narcotine although variable was primarily depressed. The degree of change ranged from 5 to 8 per cent with small to moderate and increased with larger dosages. The depressant effect of narcotine was less than one eighth that of morphine. The volume was primarily increased with all dosages. The degree of stimulation varied from 10 to 20 per cent of the original volume. This reaction was quite unlike that of morphine and was comparable to the effects produced by moderate dosages of codeine. No significant alteration of the minute volume was observed. Small doses were slightly stimulant while larger dosages were either ineffective or slightly depressant. The changes noted although suggestive are within the range of normal variation.

*Papaverine*—The respiratory rate was depressed by all dosages tested. The degree of depression increased gradually with the dosage administered and ranged from 20 to 50 per cent of the normal. The volume on the other hand was increased gradually with dosages up to 26 mg per kg. Larger doses were somewhat more depressant than morphine. The calculated minute volumes following the administration of papaverine were uniformly depressed. The degree of change varied from 5 to 18 per cent over the range of dosages tested. The depressant effects of papaverine on the respiration of the rabbit were about one-seventh that noted with equivalent doses of morphine.

#### THE EFFECTS OF PANTOPON AND THE SEVERAL OPIUM ALKALOIDS ON THE RESPIRATORY RESPONSE TO CO<sub>2</sub>

The respiration of the rabbit under the conditions of these experiments was quite inefficient in that a rapid rate and a correspondingly shallow volume was observed. The inhalation of 5 per cent CO<sub>2</sub> and 95 per cent O<sub>2</sub> resulted in a distinct

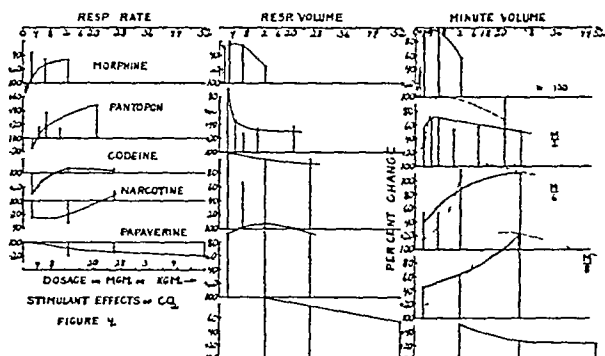


Fig 4.—This figure represents the same data for the rabbit as Fig 2 for the rat.

decrease in the rate and a more than compensating increase in the volume so that the average minute volume was increased by 50 to 80 per cent above that noted during the inhalation of 100 per cent O<sub>2</sub>.

*Morphine*—The usual rate and volume responses to CO<sub>2</sub> were distinctly altered following the administration of morphine, i e, the rate was increased and although the volume was increased the degree of change was distinctly less than in the absence of medication. This altered reaction is quite understandable when the effect of morphine alone is recalled, i e, a marked depression of rate and a relatively slight alteration of volume. In Fig 4, the respiratory rates and volumes after medication were considered as 100 per cent in order to illustrate the relative stimulant effects of CO<sub>2</sub> on the depressed center. Under such conditions the percentile change was distinctly greater after than before medication although the absolute minute volumes after medication diminished progressively with increasing dosages. The disparity between the reactions to CO<sub>2</sub> was apparent with the minimal dosage tested, increased to a maximum with 26 mg per kg and diminished with larger doses. Measurements of the actual minute volumes likewise indicate that the stimulant effects of CO<sub>2</sub> are proportionately greater after than before medication.



*Pantopon* —The respiratory reaction to  $\text{CO}_2$  after medication with pantopon was similar and did not differ significantly from that described for the one-half dosage of morphine under similar conditions

*Narcotine, Papaverine, and Codeine* —The rate and volume changes which occurred on inhalation of  $\text{CO}_2$  following medication with these agents differed in certain respects from those noted after morphine, in that the rate increase was smaller in degree and the volume was increased rather than reduced as in the case of morphine. These differences are explained by the disparity between the degrees of respiratory center depression produced by morphine on the one hand and by these minor alkaloids on the other. In spite of the observed sedative effects of codeine, papaverine, and narcotine on respiration, the sensitivity of the center to  $\text{CO}_2$  was practically unaltered and in the case of narcotine an apparent stimulation occurred

#### DISCUSSION AND SUMMARY

*Morphine* —The respiratory changes noted in the rat following the administration of morphine differ only in minor respects from those noted by Barbour and Maurer (1920). Small doses may increase the rate slightly although a mild depression is the rule. Medium doses produce a progressive depression. The volume was reduced by all dosages tested. However, the degree of change bore only a qualitative relation to the dosage administered. The minute volume was decreased by all dosages tested and the degree of change increased gradually with dosage so that with 63 mg per kg the gas exchange was only 35 per cent of normal.

The respiratory changes noted in the rabbit do not differ in any important respect from those noted by Gscheidlen (1869) or Impens (1899). Minimal doses affected the rate to a negligible degree. The depression was reflected primarily by alterations in rate in contrast to the nearly equal changes which occurred in both rate and volume in the rat. The volume was increased slightly by small doses, practically unaffected by medium to large and markedly depressed by toxic dosages. The actual minute volumes were increased with doses of 0.26 mg per kg (equivalent to 0.1 per cent of the M L D for the rabbit). The minimal depressant dosage was approximately 0.65 mg per kg body weight.

The depressant effects of morphine on the rat and rabbit are quite comparable when only changes in minute volume and the differences in the sensitivity of the two species to this alkaloid are taken into consideration. The degree of depression produced by the maximal dosages tested (1 e 13 mg in the rabbit = 5 per cent of the M L D, and 63 mg per kg in the rat = 15 per cent of the M L D) was the same (65 per cent) in each species.

The respiratory changes which occurred following the administration of narcotine, codeine, or papaverine differ only quantitatively from those noted for morphine, as reported by v Merck (1848), v Schroeder (1883) and Meissner (1913). Only quantitative differences were noted between the responses of the two species. Straub (1912) reported that narcotine alone was ineffective, but Mohrke (1921), and Rikl (1927) observed an increase in respiration in the presence of this alkaloid.

The depressant effects of codeine increased gradually to a maximum and subsequently decreased with larger doses as the reflex excitability increased. The depressant action of papaverine increased gradually to a maximum with medium

doses but further increase in dosage produced little further depression. The reaction to narcotine was of little significance.

On the basis of the degree of depression produced by the several agents tested, codeine in small to medium dosages was approximately one-fourth, narcotine in small to moderate doses was one-fourth and in larger dosage less than one eighth, while papaverine in doses up to 63 mg per kg was one-twelfth and in larger dosages approximately one-twentieth as depressant as morphine.

A direct comparison of the respiratory effects of morphine and pantopon on rats and rabbits indicates that the effects of the two agents differ only quantitatively although the responses to small doses of pantopon were more variable than after morphine. The significance of these minor differences is questionable. The pantopon effects may be entirely explained on the basis of the morphine contained. No evidence was obtained which would indicate that a potentiation of the effects of morphine occurs in the presence of narcotine as reported by Sahli (1910), Straub (1912), or Mohrke (1921). The effects produced by a combination of the residual alkaloids of opium with morphine probably represent a simple summation of the several actions as claimed by Wertheimer-Raffalovich (1910), v Zeelen (1911), Buigi (1912) and Meissner (1913).

The depressant effects of the several agents on the respiratory center as illustrated by the stimulant effects of 5 per cent  $\text{CO}_2$  before and after medication differ only quantitatively and parallel in general the changes which occur in the rate, volume, and minute volume, with the exception of narcotine and large doses of codeine. Narcotine apparently sensitizes the respiratory center to  $\text{CO}_2$  in that the stimulant effects of this gas after medication with this alkaloid were greater than normal. It is quite probable, however, that the effects of narcotine are due to both stimulation and depression since the stimulant action of  $\text{CO}_2$  diminished markedly with increasing dosages.

The efficiency of morphine, pantopon, and codeine as antidyspneic agents is probably illustrated by their effects on the respiratory reaction to  $\text{CO}_2$ . The sensitivity of the respiratory center to  $\text{CO}_2$  was reduced by all dosages of morphine or pantopon. However, in therapeutic dosages, the changes in ordinary respiration were not significant especially when the more efficient type of respiration which occurs after such medication is considered. Codeine should prove to be of value.

Toxic dosages of morphine or pantopon markedly depress ordinary respiration and distinctly decrease the sensitivity of the center to  $\text{CO}_2$ . Codeine and papaverine in large dosages were also slightly depressant but in the main, codeine, papaverine, and narcotine were convulsant in toxic dosages and the respiratory depression was of little significance.

#### CONCLUSIONS

1. The respiratory changes which occur in the rat and rabbit following medication with morphine, pantopon, codeine, narcotine and papaverine have been studied. Little difference was observed between the responses after medication when the difference in species sensitivity was taken into consideration.

2. On the basis of the degree of depression produced by the several agents, pantopon was one-half, codeine in small to medium doses one-fourth, narcotine one-fourth to one-eighth and papaverine one-seventh as depressant as morphine.

3 The depression of the respiratory center by the series studied parallels in general the observed changes in minute volume, with the exception of narcotine and large doses of codeine. Narcotine apparently sensitizes the center to  $\text{CO}_2$  but this effect decreases with increasing dosage.

4 The residual alkaloids of opium in the proportion present in pantopon do not alter the usual respiratory response to morphine to any remarkable degree. The respiratory effects of pantopon may be explained on the basis of its morphine content.

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## CHANGES IN DISTRIBUTION RATIO OF CONSTITUENTS OF BLOOD AND SPINAL FLUID IN MENINGITIS\*

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IN THE study of the changes in the spinal fluid during the progress of meningitis, it must be borne in mind that all the abnormal reactions of the tissues which constitute the investing membrane of the brain and spinal cord, and to some extent the tissues of the brain itself, may be produced by different causal agents brought to the parts by diverse channels, in other words, the invading microorganism and the mechanics of invasion may differ in various types of meningitides, but the effects are characteristically similar in the tissues affected, and they produce symptoms common to the involved tissues but modified to some extent by the nature of the invading organism and the number actually introduced. We have endeavored in this work to trace some of the common characteristics of the disturbances of physicochemical equilibria which result from the pathologic state, in order, if possible, to arrive at some definite chemical changes which may aid us in diagnosing at an early date the signs of meningeal involvement.

Animal experiments carried out by Kopetzky,<sup>1</sup> in which the intracranial pressure was artificially increased, showed that anemia of the brain could be produced and that the increased amount of cerebrospinal fluid then formed in the intracranial spaces resulted in a compressed blood supply, with interference with both the afferent and efferent vessels, and hence resulted in a lessened oxygenation of the blood in this region. It has been shown by Meyerhof and Warburg that the result of lessened oxygen tension is an incomplete oxidation of carbohydrate, so that the so-called anaerobic type of oxidation preponderates over the aerobic, and large quantities of lactic acid are produced. This phenomenon can be seen in the blood during fever, in certain forms of heart disease, and in cases of heavy and sudden exertion, where the rate of oxygen supply becomes insufficient for the total combustion of the sugars, the carbon dioxide and water.

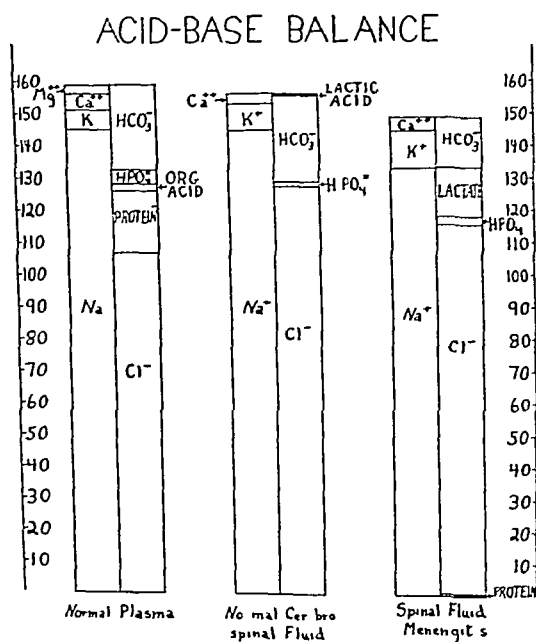
There is an increased amount of lactic acid in the spinal fluid in meningitis. The source of this lactic acid is as yet doubtful, but it is certain that the factors above mentioned tend to increase its concentration in the spinal fluid. There have been several theories that the increased lactic acid was a result of the fermentation of the sugar, which disappeared from the spinal fluid during the progress of many cases of meningitis. That this factor also may play a rôle in some cases cannot be denied, but we have, as was also shown by Killian,<sup>2</sup> found in certain cases a much increased lactic acid in the presence of a practically normal spinal fluid sugar content. There is a possibility that the disintegration of the leucocytes in purulent meningitis may be a source, because Levene and Meyer<sup>3</sup> have shown that leucocytes

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suspended in Henderson's phosphate mixture containing glucose produce glycolysis with formation of lactic acid. Again, it is a known fact that the embryonic cells of the choroid plexus have an active glycogen metabolism, so that it is not without the realm of probability that the cells of the choroid plexus may be able to actually secrete the lactic acid and concentrate it from the blood, a function that has lately been shown by one of these authors to be characteristic of the skin.

In normal spinal fluid there is a definite ratio for the relationship of spinal fluid lactic acid to blood lactic acid which never exceeds 1.0, and is usually approximately 0.6. This may even be lower in cases of exertion, fever or heart disease. In such cases, due to the presence of increased lactic acid in the blood, and an absence of specific inflammatory condition in the meninges, the ratio is lower because of the



increased blood lactic acid. In cases of true meningitis, this ratio may rise to 3 or 4 during the progress of the disease. This effect was noted by Glaeser<sup>4</sup> and confirmed by Kilham.<sup>2</sup> Glaeser found, that in two cases of epidemic spinal meningitis, the lactic acid in the spinal fluid rose to more than 150 mg per 100 c.c. This would give a ratio of over 5. In our cases as can be seen from Table I the range was between 1.5 and 3.5.

The increased lactic acid content of the spinal fluid results in several interesting consequences. The lactic acid, being a relatively strong acid, drives off  $\text{CO}_2$  from the carbonates, and hence, in cases of meningitis we should expect to find on chemical analysis of the spinal fluid a considerably lowered  $\text{P}_\text{H}$ , a decreased bicarbonate content and an increased lactic acid. This is found to be exactly the case.

Another effect of the increased lactic acid found in spinal fluid in meningitis can be predicted on theoretical physical chemical grounds, which experimental re-

TABLE I

TYPE OF CASE	PH	CO. VOLUME PER CENT	CO	LACTIC ACID		PROTEIN MG PER 100 CC	Na CC N/10	N <sub>t</sub> MG PER 100 CC	K CC N/10	K MG PER 100 CC	BLOOD LACTIC ACID MG PER 100 CC	RATIO SPLA/BLA	CL MG PER 100 CC	CL N/10	BLOOD PLASMA CL MG PER 100 CC	BLOOD PLASMA CL/BL FLUID GRADIENT MG/100 CC
				MG PER 100 CC	CC N/10											
Strep hem meningitis	6.9	49.0	22.2	22	3.6	208	132	304.7	9	17	20.9	1.05	350	98.5	348	+2
Strep hem meningitis	6.9	50.2	22.6	54	6.0	202	122	281.7	7.5	14.2	28	1.92	340	93.0	364	-24
Meningitis	6.9	43.8	19.8	120	13.3	144	145	333.5	11.1	27	34	3.54	372	102	356	+16
Meningococcus meningitis	6.9	47.8	21.4	34	3.9	94	137	310.5	11.2	21.3	21	1.62	361	107	361	+20
Tb meningitis	7.1	44.2	19.8	84	9.3	235.6	127	293	14.7	28	26	3.00	362	102	364	-2
Tb meningitis	7.3	51.9	22.9	84.5	9.4	252	137	316	11.2	21.3	26	3.23	374	100	359	-5
Tb meningitis	7.4	56.6	25.4	84	9.3	267.7	144	332	12.6	21	28.1	2.80	369	102	363	+6
Meningitis mumps	7.0	43.6	19.6	75.5	8.1	118	136	314		18	29	2.6	341	99	347	-6
Meningitis	7.2	50.7	22.7	58.2	6.5	109	137	317		19	28	2.1	342	93	351	-19
Meningitis	7.2	50.1	22.6	63.0	7.0	284	139	320		21	23	2.7	346	99.3	349	-3
Strep hem meningitis	7.0	40.4	18.2	67.2	7.52	212	114	309		18	19	3.5	312	93	359	-27
Mastoiditis	7.5	60.0	27	21	2.3						20	1.05	412	116	370	+42
"Normal"	7.52	63.2	28.5	9	1.0		110	324	9	17	14	0.64	342	124	365	+77
Pneumonia	7.49	62.1	28	18	2.0		111.5	329	7.9	15	14	0.41	303	113	361	+39

sults confirm, though not entirely quantitatively. Between the plasma in the circulation and the body fluids outside the circulation a Donnan equilibrium must exist. The analyses of Loeb, Atchley and Palmer<sup>4</sup> have shown that in the case of edema fluid, the membranes separating the circulating blood from the transudate behave differently from the membranes separating the erythrocytes from the plasma in that  $\text{Na}^+$  and  $\text{K}^+$  are readily interchanged, and under normal conditions proteins do not pass through. The corpuscular membrane is impermeable to  $\text{Na}^+$  and  $\text{K}^+$  as well as protein. They found that the Donnan equilibrium can be applied to measure the distribution of the ionic constituents between plasma and spinal fluid, as well as between corpuscles and plasma. Hamilton<sup>5</sup> has determined the distribution ratios between blood plasma and spinal fluid. He found that the spinal fluid maintained a Donnan equilibrium with the blood plasma less regularly than edema fluid, but that the same general laws could be applied. Eighteen of his patients were epileptics, in whom there was an increase of lactic acid in the spinal fluid. He did not, however, make allowance for this factor which might have been one of the disturbing factors to which he refers. From the fundamental postulates of the Donnan equilibrium, we have

$$\begin{aligned}(\text{Na}^+)_s &= (\text{Cl}^-)_s - (\text{P}_1^-)_s \\ (\text{Na}^+)_{sp} &= (\text{Cl}^-)_{sp}\end{aligned}$$

where  $\text{P}_1$  is the concentration of the protein in the serum bound as negative ion to base, and  $\text{Na}^+_s$  and  $\text{Na}^+_{sp}$  and  $\text{Cl}^-_s$  and  $\text{Cl}^-_{sp}$  are in serum and spinal fluid respectively.

The normal spinal fluid is practically protein free so that in accordance with the Donnan equilibrium we have

$$r = \frac{(\text{Na}^+)_s}{(\text{Na}^+)_{sp}} = \frac{(\text{Cl}^-)_{sp}}{(\text{Cl}^-)_s + (\text{P}_1)_s}$$

$\text{Cl}^-_{sp}$  would have to be greater than  $\text{Cl}^-_s$ . This is normally found to be the case. In cases of meningitis where we have an increased protein content of the spinal fluid, this would tend, in accord with the ratios expressed above, to lessen the chloride content of the spinal fluid and more nearly approximate it to that of the serum. This influence cannot be the paramount one, even in the severest cases of meningitis, because the maximum amount of protein ever found is less than one milliequivalent per 100 cc. But another factor plays a rôle here

$$\frac{(\text{Cl}^-)_s}{(\text{Cl}^-)_{sp}} = \frac{(\text{HCO}_3^-)_s}{(\text{HCO}_3^-)_{sp}} = r$$

When the lactic acid, which is a stronger acid than carbonic enters, the result is a driving off of  $\text{CO}_2$  from the spinal fluid so that the ratio given above does not hold momentarily and  $\text{Cl}^-$  migrates back into the blood stream in an effort to restore the normal distribution ratio. Hence, in all cases of meningitis, there is a significant lowering of the spinal fluid chloride and a decrease in the gradient between spinal fluid chloride and plasma chloride. This point is of diagnostic value.

If we were to add to 1 liter of perfectly pure water of  $P_H$  7.0, 1 cc of 0.01 N HCl the resulting solution would be about  $P_H$  5.0 and very toxic to certain bacteria. If, on the other hand, we were to add the same amount of acid to 1 liter of a standard beef infusion of  $P_H$  7.0, the resulting change of  $P_H$  would be scarcely appreciable. This power of resistance to change in  $P_H$  with added acid is known as the buffer power of the solution. If acid produced by the catabolic processes of the body is added to blood, there is extremely little change in  $P_H$  because of the action of the hemoglobin and the serum proteins which, acting as buffers, stabilize the reaction. These are missing from the spinal fluid, which then becomes dependent on the carbonates for any real buffer power. It can be seen that, since the real stimulus of the respiratory center is due to changes in the  $P_H$  content of the blood, and a change in  $P_H$  of 0.1 has an enormous effect on the respiratory center, that the need for stability of reaction of the actual fluid, which probably bathes the respiratory center, is very great. The carbonates are the only possible buffer. It has been shown that the maximum buffer effect of a weak acid and its salt is attained when this acid and salt are present in equimolecular quantities. In blood the carbonates play a subsidiary rôle in keeping the hydrogen ion content stable. There is normally present in the blood about 20 equivalents of  $\text{NaHCO}_3$  but only 1.2 equivalents of carbonic acid. Hence the carbonate buffer value must be very low. In cases of meningitis, there are about 8 equivalents of carbonic acid in spinal fluid released by the lactic acid. Hence the ratio instead of 20/1.2 becomes 12/8.5 which is a much nearer approach to maximum buffer action. The analogy to the low bicarbonate content of the blood and somewhat depressed  $P_H$  in the terminal stages of diabetic coma due to the accumulation of  $\beta$  oxybutyric acid is at once apparent. However, there is a fundamental difference, because the accumulation of the  $\text{CO}_2$  at the expense of the plasma bicarbonate results instantly in such a powerful stimulus for the respiratory center that the breathing rate is accelerated and the blood passes through the lungs so much more often that the increased oxygenation results in a  $\text{CO}_2$  plasma content that is even lower than normal and the buffer action is depressed. With the spinal fluid there is no such passage through an aerating mechanism and the buffer power of the spinal fluid is increased by the accumulation of carbonic acid. The lactic acid itself cannot function as a buffer since its maximum buffer action is only exerted around  $P_H$  4.0 and at the  $P_H$  of the spinal fluid its buffer action is practically 0.

*Summary*—In the spinal fluid of patients suffering from meningitis there is a decrease in the spinal fluid chloride content and a decrease in the spinal fluid chloride, blood plasma chloride ratio which can be predicted from the postulates of the Donnan equilibrium. There is an increase in the lactic acid content of the spinal fluid and a lowering of the spinal fluid carbonate, blood plasma carbonate ratio. This leads secondarily to an increase in the buffer power of the spinal fluid carbonates and helps maintain the spinal fluid nearer its physiologic  $P_H$ .

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## STUDIES ON ANTIPEPSIN WITH A NEW QUANTITATIVE METHOD FOR ITS DETERMINATION\*

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THE predilection of chronic ulcerations of the gastrointestinal tract for the stomach and duodenum suggests that an important factor to be considered in the etiology of peptic ulcer is some peculiarity in the physiology of the stomach. This organ is the only part of the alimentary canal which secretes hydrochloric acid and pepsin. It is doubtful whether a high free hydrochloric acid content is of much importance in the etiology of peptic ulcer, for there are too many patients suffering with peptic ulcer whose gastric contents show no free hydrochloric acid. If peptic ulcer is a local digestive process of the gastric wall, there must be some explanation for the occurrence of this local digestion in the stomachs of some people and not in others. One theory states that certain people are deficient in some inhibitory agent which normally prevents the pepsin in the stomach from digesting the gastric wall. The blood, in flowing through the tissues of the stomach, may there deposit or excrete this protective antipeptic substance.

Weinland<sup>1</sup> extracted a substance from *Ascaris* worms which would neutralize and inhibit the activity of pepsin, thus explaining the passage of parasitic worms through the stomachs of their hosts unharmed. Earlier workers, including Sachs,<sup>2</sup> Schwarz,<sup>3</sup> Beitzke and Newberg,<sup>4</sup> and Katzenstein,<sup>5</sup> reported positive results in the attempted demonstration of antiferments in the blood sera of animals. Later contributors to the subject, as Burge,<sup>6</sup> Bayliss,<sup>7</sup> Orator,<sup>8</sup> Keiser,<sup>9</sup> and Hilarowicz and Mozolowski,<sup>10</sup> maintain that no one has yet proved the existence of antipepsin.

This study was undertaken to work out a method which would be satisfactory in the detection and possibly in the quantitative estimation of antipepsin, and to ascertain if such a substance could be demonstrated in the sera and tissues of normal animals and animals subjected to a course of injections with pepsin.

### METHODS

- 1 The principle of the method finally evolved is the peptic digestion of albumin. Ten small tubes are set up in a rack, and to each are added fresh preparations of 0.7 per cent solution of dried egg albumen, 0.0005 per cent stock solution of Merck's C. P. pepsin in 10 per cent glycerine, 2.5 per cent hydrochloric acid, 0.1 cc of a 0.04 per cent solution of serum, and water, in the quantities indicated in

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Table I The solubility of preparations of egg albumen vary. In this procedure, the albumin solution must contain 0.7 per cent protein by chemical analysis. The control set up is exactly like the other, except that instead of containing the serum to be tested, it has either the serum of normal, noninjected animals, or a quantity of additional albumin solution whose protein content is exactly the same as that of the serum being tested.

The system is placed in an incubator at 37.5° C. After one hour has elapsed, the tubes are removed, and to the contents of each is added one cubic centimeter of 4.5 per cent trichloroacetic acid. The undigested protein is immediately precipitated as a white cloud, the intensity of which varies inversely with the degree of peptic digestion of the protein.

The measurement of peptic digestion may be done by either visual or chemical methods. In the former, the tubes are held up to the light, and the cloudiness in the control is compared to that of the tube which contains the serum in question. A nephelometer would undoubtedly improve the accuracy of this method. Chemical estimations are most accurate and are made by measuring the amount of nonprotein nitrogen in each tube by an adaptation of the Folin-Farmer<sup>11</sup> method for total nitrogen determinations in urine.

2 In order to determine the antipeptic effect residing in the tissues of the animals which had received injections of pepsin, two methods were devised which are adaptations of the above procedure. The principle of one of them is the direct digestion of the tissue in question by pepsin. Test tubes containing water, 2.5 per cent hydrochloric acid, and amounts of pepsin to make up a final concentration of this substance varying from 0.0002 per cent to 0.0001 per cent, are set up. One gram each of fresh liver, muscle, duodenum, and stomach mucosa from 4 pepsin-injected animals and two non-injected animals (controls) is placed in the test tubes, each tissue being tried in three different concentrations of pepsin. After an hour at 37.5° C, 4.5 per cent trichloroacetic acid is added, and nonprotein nitrogen determinations made on the filtrates of the contents of each tube.

3 The third procedure is based upon the effect of glycerine tissue extracts on peptic digestion of albumin. The set-up is similar to that given in Table I. In-

TABLE I

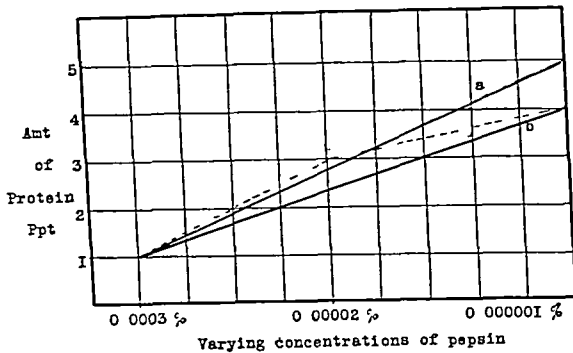
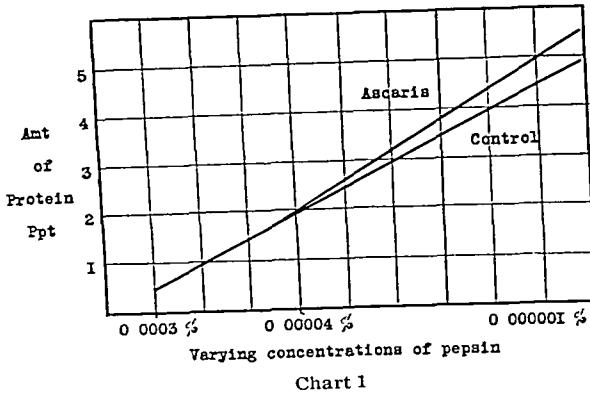
	1	2	3	4	5*	6	7	8	9	10
Albumin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Serum	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Pepsin	2	12	0.6	0.24	0.12	1	0.75	0.3	0.15	0.1
HOH	0.15	0.95	1.55	1.91	2.03	1.15	1.4	1.85	2	2.05
Total	3 c c	3 c c	3 c c	3 c c	3 c c	3 c c	3 c c	3 c c	3 c c	3 c c

\*In tubes 5-10 a 0.00004 per cent pepsin solution is used.

stead of serum, however, 0.2 c c of extracts obtained by extracting 5 gm. of liver, muscle, duodenum, and stomach mucosa for twenty-four hours at room temperature with 75 c c of 65 per cent glycerine, are used. Nonprotein nitrogen determinations are made on the filtrates of the contents of each tube.

# RESULTS

1 To prove the sensitivity of our method for the detection of anti-pepsin, it was necessary to test it on some substance known to contain anti-pepsin. Accordingly 3 gm. of dry, powdered *Ascaris* was extracted with 50 c.c. of 50 per cent glycerine for eighteen hours, filtered, and the filtrate diluted one to four. The procedure described under methods (1) was carried out, using 0.2 c.c. of this diluted material instead of the serum. Results are indicated in Chart 1, and demonstrate that when the pepsin concentration falls below 0.00004 per cent, there is definite inhibition of peptic activity in the tubes where the *Ascaris* extract was used.



\* Dotted line represents curve before injections  
( control curve identical )  
a Serum of animal after injections  
b Serum of control animal to a

2 The sera of twelve normal, healthy, rabbits were tested by the procedure described under methods (1). Using both visual and chemical methods of measurement, it was found that half the animals showed an anti-peptic activity of the blood serum and the other half did not.

3 Two animals which showed no anti-peptic activity of the blood sera were given intravenous injections of 0.2 gm. of pepsin (Merck, C. P.) every five days for one hundred and fifty days. After a few weeks, the sera of both showed anti-peptic activity. Chart 2 gives the results of the serum tests on one animal before and

after injections. Marked antipeptic activity occurs when the pepsin concentration is less than 0.0003 per cent. Seventy-seven per cent of nonprotein nitrogen determinations confirmed the results.

Five animals were injected intravenously for one hundred and fifty days every five days, beginning with 0.01 gm. and increasing the dosage by 0.025 gm. each time. Three of these animals had shown no antipeptic activity of the blood sera before injections, and developed it after several injections. Seventy-six per cent of the chemical determinations confirmed these results.

4. Eighty-three per cent of the nonprotein nitrogen determinations obtained by using the method of direct digestion of tissue by pepsin for estimating how much antipepsin may be detected in the tissues of animals, demonstrated, in 2 rabbits, that the livers of the pepsin injected rabbits were antipeptic as compared with the livers of noninjected animals. With the other tissues, duodenum, muscle, and stomach mucosa, only 60 per cent of the determinations showed antipeptic activity.

5. The results obtained by finding the effect of glycerine extracts of tissues of 4 animals on peptic digestion of albumin, showed that 83 per cent of the nonprotein nitrogen determinations demonstrated a high antipeptic activity of the muscle extracts, and a low one (60 per cent) with the other tissue extracts.

#### DISCUSSION

In the methods described, the  $P_H$  is well controlled. This is important, for changes in the reaction of a medium will definitely alter the activity of the enzyme contained. In fact, Bayliss<sup>11</sup> and Popper<sup>12</sup> concluded that all positive reports on antipepsin are due to the unsatisfactory control of the hydrogen ion concentration. In the set-up described, although the reaction might possibly vary slightly from week to week, it could not vary between the contents of any given control tube and the contents of the tube which is being tested. The presence of a strong mineral acid (HCl) tends to keep the  $P_H$  constant. No salt is introduced into the solutions used, thus ruling out the possibility that it is responsible for any inhibition of peptic activity, as has been asserted by Hamburger.<sup>13</sup>

The fact that the same amount of protein is present in the solutions of the control and test tubes prevents the supposition that an antipeptic effect is due to the absorption of pepsin by the protein. Trichloroacetic acid was chosen as the precipitating agent, because in the concentration used, it will precipitate the protein out of a solution of partially digested protein without carrying down the protein split products.<sup>14</sup>

The results with the ascaris extract prove that the test is sensitive in the estimation of antipepsin. Only one-half the normal, uninjected rabbits showed any antipepsin activity of the blood serum. There may be a variation in antipepsin in different animals, or quite possibly, the substance may be present in such small amounts normally that the test devised is not sufficiently sensitive to detect it. The test is sensitive enough, however, to detect the development of antipeptic activity of the blood stream when a course of injections with pepsin has been given. Six animals showed the presence of antipepsin in the blood stream after intravenous injections of pepsin.

Results seem to point to the liver as being the tissue where most of the antipepsin resides, although the extract of liver was not very antipeptic, due perhaps to the

influence of the new factor, namely, glycerine extraction. The liver being the most active site of antibody formation, this would strengthen the belief that the antipeptic quality detected in the blood stream and tissues of the animal used, is an antibody-like substance. It is certainly, however, not produced in anything like the quantities which antibodies are usually produced. Kolmer<sup>15</sup> believed that there are anti-antiferments which prevent the accumulation of too much antiferment. It is felt that if the methods described were used to determine the antipeptic activity of the blood sera of patients suffering from peptic ulcer, results might be obtained which would have some bearing on the problem of the etiology of peptic ulcer.

#### CONCLUSIONS

- 1 A new method is presented for the detection and relative quantitative estimation of antipepsin.
- 2 Rabbits, injected intravenously with pepsin over a period of time, showed the development of an antipeptic activity in the blood sera.
- 3 This antipeptic activity is due to a real antibody, namely, antipepsin.
- 4 The liver is the organ with the largest store of antipepsin.

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# THE EFFECT OF SHORT RADIO WAVES ON THE BIOLOGIC ACTIVITIES OF SOME BACTERIAL SPECIES\*

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THE discovery that an increase of temperature was produced in substances or animal bodies exposed to short radio waves, has stimulated investigation regarding the possible application of this new knowledge to medical therapy. From the experiments undertaken data have also accrued, which are of more academic interest.

## HISTORICAL REVIEW

Schereschewsky's early work on mouse tumors<sup>1</sup> was followed by Hosmer's<sup>2</sup> report on the effect of heating various electrolytes under different conditions. She also observed the resultant tissue changes when rats were heated by exposure to short radio waves. Pariseau<sup>3</sup> observed that egg could be coagulated by the action of the waves acting in very definite sites in the field between the plates. Kähler found that the action on pyramococci was strictly comparable to that produced by ordinary means of heating these animals. MacCreight and McKinley<sup>4</sup> killed mice by exposure to short radio waves. Carpenter and Page<sup>5</sup> reported upon their experiences in exposing human subjects to short radio waves. Later, Carpenter and Bork<sup>6</sup> showed that increased temperature in rabbits produced by short radio waves could prevent experimental syphilis. They, as well as other investigators, observed that the cells of different portions of the body did not respond in a uniform manner. Thus, the temperature of one organ, such as the liver, was greater than that of skeletal muscle. The physiologic and biochemical changes wrought by exposure to a high frequency field have been described by Knudson and Schaible,<sup>7</sup> while morphologic changes were studied by Jacobsen and Hosoi.<sup>8</sup> McLennan and Burton<sup>9</sup> demonstrated the selective heating by this means on pieces of dead tissue.

Szymanowski and Hicks<sup>10</sup> have recently analyzed exhaustively the literature relative to the effects of radiotherapy; they have also indicated the results of their own experiments on exposure of thin films of bacterial toxins; they believed that the toxicity or the products was reduced some what and that the effect was not due to heat created by the radio waves, since provision had been made to exclude that factor. A further review of the current knowledge regarding this subject has been given by Hemingway and Stenstrom.<sup>11</sup>

Ross,<sup>12</sup> working in this laboratory with the Rous chicken sarcoma, has not been able to confirm Schereschewsky's and Schereschewsky and Andervont's<sup>13</sup> early experience with the mouse tumor. Ross found that exposure of chickens inoculated with the Rous sarcoma did not retard, but rather enhanced the growth of the sarcoma.

## EXPERIMENTS

The equipment employed for obtaining the heating effects about to be described consisted of a high frequency heater as shown in Fig. 1, which was the gift of the General Electric Company of Schenectady and the Canadian General Electric Company of Toronto, arranged through the kindness of Dr. Whitney. A wavelength of 30 meters as registered on a wavemeter was consistently used in all the experiments herewith outlined. The plates were set at a distance of eleven inches apart. When the object exposed to the effect of the machine was elevated above the

\*From the Research Laboratories of the Sub-Department of Paediatrics, University of Toronto and the Hospital for Sick Children under the direction of Alan Brown, M.D.  
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floor between the plates, it was supported on a wooden stand at a height of  $9\frac{1}{4}$  inches, as shown in Fig 1 Both cultures and animals were placed parallel to the field unless otherwise indicated

#### EFFECT OF SHORT RADIO WAVES ON BIOLOGIC AND SEROLOGIC CHARACTERISTICS OF BACTERIA

*On Pigment Production and Colony Form*—*B. pyocyaneus*, spread on a plain nutrient agar plate, P<sub>H</sub> 7.4, was incubated for eighteen hours The cover was removed, the plate secured on the wooden stand in a vertical position at right angles to the plane of the field between the plates Exposure was continued for one and one-half hours Colonies were transferred from this plate to a fresh plate After incubation no diminution in the power to produce the characteristic pigments of *B. pyocyaneus* was noted, as the culture possessed the same intensity of greenish yellow color as the original culture

An eighteen hour nutrient broth culture of *B. prodigiosus* was placed in a sausage-shaped pyrex T-tube, such as McLennan and Burton<sup>11</sup> used, and heated for fifteen minutes in the machine The temperature taken at the end of that time was 62° C, an increase of 30° C having taken place in the fifteen minutes A bacteriologic loopful of the culture was streaked on a plain nutrient agar plate Abundance of pigment was shown after the plate was incubated, and the "smooth" colony form had not changed

*B. prodigiosus* was also heated in a pyrex T-tube surrounded by a pyrex jacket In the outer chamber was an electrolyte consisting of sodium chloride at a concentration of 0.0045 gm molecules per liter as shown by McLennan<sup>14</sup> and Burton to give an optimum effect The temperature rose 32° C in a fifteen-minute exposure Again no change was noted in the effect produced in the culture either as to pigment production or colony form

*On Biologic Characteristics*—*Salmonella murioitidis* was spread on a nutrient agar plate and treated as the *pyocyaneus* and *prodigiosus* cultures for one and one-half hours Colonies were transferred from this plate to a fresh agar plate and to agar slopes The "smooth" colony form was retained Indol and motility tests, the gas producing power in carbohydrate medium, the action on litmus milk the ability to reduce lead acetate and to produce acid in medium containing various carbohydrates were then examined The cultures selected from the plate exposed to the action of the 30 M radio waves had neither lost nor gained characteristics which differed in any respect from those possessed by the original culture

*S. murioitidis* was not killed when heated for twenty-five minutes in the machine the final temperature reached at the end of that time being 80° C

*On the Antigenu Factors of Bacilli*—A broth culture of *S. murioitidis* was subjected to a 30 meter wave for nine hours, at the end of which exposure the temperature of the culture was 41° C The culture was living The suspension was centrifuged resuspended in 0.85 per cent sodium chloride P<sub>H</sub> 7.0, and employed as the antigen for the production of an agglutinating serum in a rabbit (serum A) The results obtained by using this serum are compared with those which were given by an agglutinating serum (serum B) the antigen for which was prepared by heating in a water-bath at 56° C for one hour prior to injection (Table I) The sera were tested with fresh unheated eighteen-hour suspensions of *S. murioitidis* with a sus-

pension heated at  $60^{\circ}\text{C}$  for one hour in a water-bath, and with a suspension heated for one hour at  $100^{\circ}\text{C}$

From Table I it is to be noted that there is no essential difference in the results. Serum A apparently had a higher titer than Serum B. Similar agglutinins<sup>25, 26</sup> were present in both sera. Exposure to heat produced by short radio waves ap-

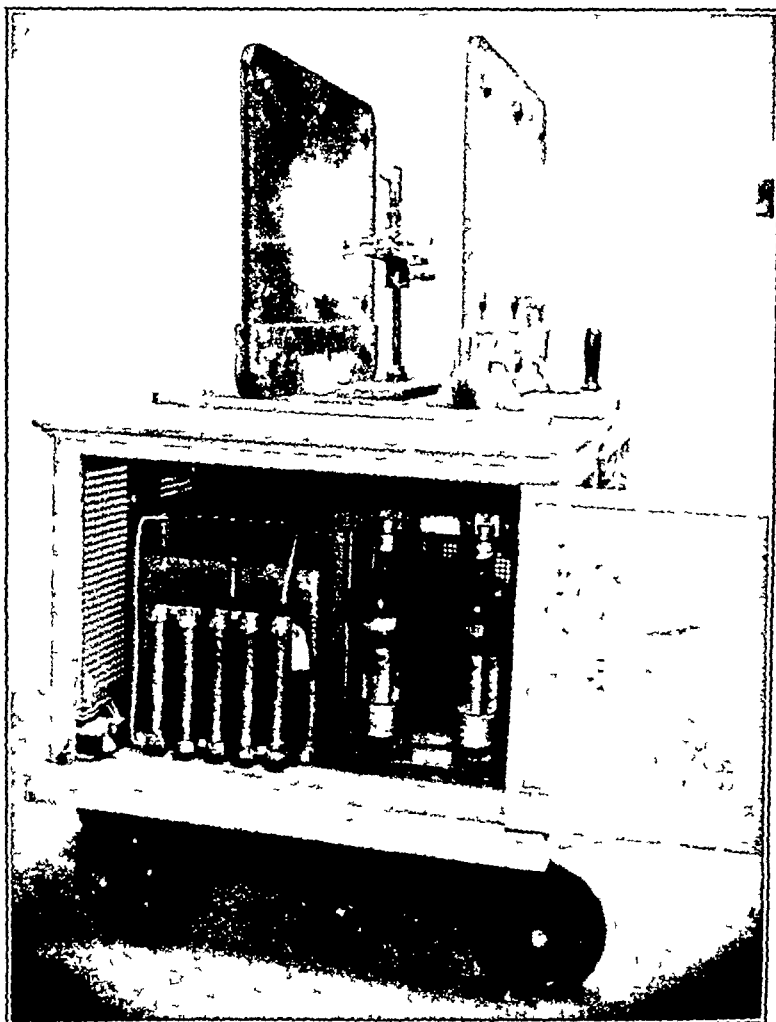


Fig 1

parently has not influenced the agglutinin antigenic factors of bacteria as far as these experiments show

#### PNEUMOCOCCUS EXPERIMENTS

An eighteen-hour broth culture of a strain of *Pneumococcus*, Type II, which was fully virulent, having been mouse-passed just before use, was employed in these experiments. A temperature of  $41^{\circ}\text{C}$  was attained by heating one portion of the culture in a water-bath for twenty-five minutes (A). A second portion was



TABLE I  
AGGLUTINATION TESTS USING SERA PREPARED WITH ANTIGEN HEATED BY 30 M RADIO WAVES,  
AND WATER BATH HEATED ANTIGEN\*

SERUM	CULTURE	SERUM DILUTIONS								
		40	80	160	320	640	1280	2560	5120	10240
Serum A Antigen Exposed to 30 M Radio Waves for 9 hours	Unheated	4	4	4	4	4	4	4	2	0
	Heated at 60° C	4	4	4	4	4	4	2	1	0
	Heated at 100° C	3	3	2	2	2	1	0	0	0
Serum B Antigen Heated in Water bath at 56° for one hour	Unheated	4	4	4	4	2	1	0	0	0
	Heated at 60° C	4	4	4	2	1	0	0	0	0
	Heated at 100° C	2	1	0	0	0	0	0	0	0

\*4 Complete agglutination  
3 Almost complete agglutination  
2, Partial agglutination  
1 Very little agglutination  
0 No agglutination

heated in the machine for twenty-eight minutes, the final temperature being 41° C (B) Each of five mice was injected intraperitoneally with 0.1 c.c. of Culture A, five other mice received 0.1 c.c. each of Culture B. A third lot of five mice received a similar injection of unheated culture and were then exposed to 30 M waves for five minutes (C). A control group of five mice (D) received a similar injection of unheated culture. No temperatures were taken.

All animals which received Culture B and D died (Table II, experiment I), two belonging to Group C died, the remainder surviving until disposed of ten days later. None of Group A died. Either the virulence was much attenuated by the water-bath heating, or the culture was not living. Cultures identical with the injected culture were isolated from the heart's blood of the autopsied mice as identified by bile solubility.

This experiment was repeated (Table II, Experiment II). The water-bath heating of the culture was continued for twenty minutes and the final temperature was 40° C. The culture heated in the machine was exposed for 30 minutes, but the final

TABLE II  
PNEUMOCOCCUS EXPERIMENTS

EXPER. NO.	CULTURE WATER BATH HEATED (A)		CULTURE 30 M WAVE HEATED (B)		UNHEATED CULTURE MICE EXPOSED TO 30 M WAVE (C)		UNHEATED CULTURE CONTROLS (D)	
	NO MICE	NO DEAD	NO MICE	NO DEAD	NO MICE	NO DEAD	NO MICE	NO DEAD
I	5	0	5	5	5	2	5	5
II	5	5	5	5	5	5	5	5
III	0	0	0	0	5	3	5	5
IV	3	3	3	3	0	0	3	3

temperature was  $34^{\circ}\text{C}$ . All animals in the four divisions died within forty-eight hours.

Ten mice injected with pneumococcus were divided into two lots of five each, one lot was exposed to 30 M waves for two minutes, the control lot was not so treated (Table II, Experiment III). Of the test lot, one mouse died immediately after injection, and a second just after having been exposed to the action of the machine. The three which remained and the five in the control group were dead in forty-eight hours. Pneumococci were isolated from the heart's blood.

A fourth experiment of a similar nature was carried out with nine mice divided into three lots of three each. One lot was injected with culture heated in water-bath to a temperature of  $42^{\circ}\text{C}$ , the second lot, culture heated in the machine for one hour to a temperature of  $41^{\circ}\text{C}$ , the third with unheated culture. All animals died, and again pneumococci were isolated from the heart's blood.

Pneumococcus did not lose its virulence for mice when exposed to 30 M waves for the length of time indicated, which caused an increase in the temperature of the culture to the degree noted. Mice which have received a lethal dose of pneumococcus and were immediately exposed to 30 M radio waves, developed their infection and died as rapidly as did the control animals which received unheated culture and were not exposed to radio waves.

Kapelow and Holden,<sup>17</sup> carrying out analogous experiments with herpes virus, found that radiotherapy had no effect on the virulence of the virus.

#### VITAMIN DEFICIENT ANIMALS

Experiments were carried out using rats which were on a vitamin D deficient diet, with a control group receiving vitamin D in a diet which included 12½ per cent vitamin D bread. A small amount, 0.08 c.c., of an eighteen-hour broth culture of *Salmonella muiotitis* was fed by mouth. This volume was sufficient to cause death in such animals fed in this manner, as determined by Robertson and Ross<sup>18, 19</sup> in their experiments on resistance to infection in rats on a vitamin deficient diet. In the first experiment the animals were given an exposure of about five minutes three days after being fed the culture. The infection is established by that time, as ascertained by Robertson and Ross.<sup>19</sup> In the second experiment the animals were not exposed until six days after the culture was fed, when the duration of exposure was from three to eight minutes.

While the number of animals in the two experiments (Table III) was small, it is seen that more vitamin D deficient rats died from the infection after exposure to the effect of short radio waves than did those not exposed, or did the animals whose diet was not deficient in this respect.

An eighteen-hour broth culture of *S. muiotitis* was heated in a water bath for two hours at  $40^{\circ}\text{C}$ , which temperature failed to kill the culture. A similar culture was heated to  $35^{\circ}\text{C}$  by 30 M radio waves. One-tenth of a cubic centimeter of these cultures was fed to rats suffering with vitamin B deficiency. Of the five animals fed the radio-wave heated culture, all died, while none of the five rats fed the water-bath heated culture died. The  $40^{\circ}\text{C}$  temperature of the latter culture may have attenuated its virulence more than the  $35^{\circ}\text{C}$  temperature obtained by radiotherapy.

Other experiments were carried out with young vitamin B deficient rats in which the culture was fed, after which the animals were exposed to the 30 M wave

TABLE III  
EFFECT OF 30 M RADIO WAVES ON INFECTION IN VITAMIN D DEFICIENT RATS

	D DEFICIENT				CONTROLS (+ VITAMIN D)			
	EXPOSED		NOT EXPOSED		EXPOSED		NOT EXPOSED	
	NO RATS	NO DEAD	NO RATS	NO DEAD	NO RATS	NO DEAD	NO RATS	NO DEAD
I	2	2	2	1	2	1	2	*2
II	3	2	2	0	3	**1	2	1
	5	4	4	1	5	2	4	3

\*Died in 72 hours No *S. murietitis* isolated

\*\*Died while being rayed No *S. murietitis* isolated

The controls in this group were supplied the vitamin in the form of yeast. The animals were fed by mouth 0.08 c.c. of an eighteen-hour broth culture of *S. murietitis* by the same method as was used with the vitamin B deficient rats. The rats in both test and control group were then given one exposure to the 30 meter radio waves for about eight minutes. All the vitamin deficient animals were dead within seventy-two hours, while a number of the controls survived. Of the controls which died, death did not occur before the end of five days, and was delayed in one animal for thirteen days. *S. murietitis* was recovered from the heart blood.

No unusual phenomena were produced by heating the bacterial culture by radio waves, which could not also be the result of more ordinary methods of heating. If this were not so, long continued exposure of the bacteria which resulted in a sub-lethal temperature would have ample opportunity to bring about such changes.

The exposure of animals which had been receiving diets deficient in vitamin B, as of those whose diet had been deficient in vitamin D, does not curtail to any significant extent the acute infection produced in such animals by the means described.

Experiments undertaken with greater numbers of animals under conditions other than those outlined in this report might produce different results. These experiments indicate, however, that acute infections are not aborted by radiotherapy.

#### SUMMARY

- 1 Heat generated by short radio waves had no effect on the colony form, pigment production, the biologic or some agglutinin antigenic properties of bacteria.
- 2 The virulence of bacterial species was not attenuated by radiotherapy.
- 3 Short radio waves did not influence acute infections (such as pneumococcus may produce) to which mice are susceptible. Neither did exposure to radiotherapy curtail a per os infection in vitamin deficient white rats.

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## STUDIES ON THE NORMAL BLOOD III THE GUINEA PIG\*

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THERE is available little literature on the normal blood pictures of the animals which are commonly used in laboratory investigations. Experimentation often requires a knowledge of the normal hemoglobin percentage, the normal number of red cells and the normal number of white cells and their distribution. We have been interested here during the last three years in making such studies on the animals which are more commonly used in the laboratory. I have<sup>1</sup> published a detailed account of a study on the blood of the normal dog.

It was the purpose of the work herein reported to determine in normal animals of both sexes and of different ages

- a. The hemoglobin percentage
- b. The erythrocyte number
- c. The total and differential white blood cell counts

All animals were kept in the laboratory for a period of two weeks before beginning the experiments. The diet consisted of hay, oats, cabbage, lettuce, carrots, and grass. The animals appeared to be normal in every respect and exhibited no nervous reactions during the obtaining of blood for the counts.

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The usual clinical methods were employed throughout the work. For making the leucocyte count the Leitz hemocytometer with Neubauer ruling was used. The dilution pipette afforded a 1 to 20 or 1 to 10 dilution. Turk's solution was the diluting medium. The blood was drawn to 0.5 mark and the diluting solution immediately drawn up to 11 mark (1 to 20 dilution). In counting, the cells in four large squares of sixteen small squares each were counted and their sum multiplied by fifty.

The Leitz hemocytometer with Neubauer ruling was also used in the erythrocyte determinations. The diluting pipette afforded a dilution of 1 to 200 or 1 to 100. Hayem's solution was the diluting medium. The blood was drawn up to the 0.5 mark and immediately diluted to the 101 mark (1 to 200 dilution). The cells in five squares of sixteen smaller squares each (80 small squares) were counted and the sum multiplied by 10,000.

The cover glass method was employed in making blood smears. The smear was dried in air and stained with Wright's stain for one minute. Distilled water was placed on the smear for four minutes, after which the preparation was rinsed under tap water and allowed to dry. The cells were studied under oil immersion.

In the differential counts, all neutrophils, basophils, and eosinophils were grouped as polymorphonuclears. There were some differences among the various polymorphonuclears, but the granules in no case stood out as they do in the human basophils and eosinophils. The unquestionable neutrophils had a heavily stained irregular nucleus with a pale or almost colorless cytoplasm, while the cytoplasm of the polymorphonuclears was somewhat granular, but the granules were too small, and the color too indefinite to class them as eosinophils or basophils.

GUINEA PIG 1—MALE, THIRTY WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES		
				P M N PER CENT	LARGE SMALL PER CENT	TRANS PER CENT
First week	79	5,170,000	13,600	64	35	1
Second week	80	5,520,000	13,400	52	47	1
Third week	80	4,930,000	13,900	58	42	0
Fourth week	78	4,970,000	14,000	60	38	2
Fifth week	79	4,830,000	15,800	57	39	4
Sixth week	80	5,140,000	14,600	54	44	2
Average	79	5,093,000	14,200	57	41	2

GUINEA PIG 2—FEMALE, THIRTY WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES		
				P M N PER CENT	LARGE SMALL PER CENT	TRANS PER CENT
First week	77	5,300,000	14,600	63	35	2
Second week	78	5,380,000	14,000	63	37	0
Third week	76	5,141,000	13,400	64	35	1
Fourth week	77	5,090,000	14,400	67	30	3
Fifth week	78	5,140,000	14,400	65	33	2
Sixth week	77	5,150,000	14,000	56	44	0
Average	77	5,200,000	14,100	63	35	1

GUINEA PIG 3—MALE, THIRTEEN WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES		
				P M N PER CENT	LARGE SMALL PER CENT	TRANS PER CENT
First week	73	4,810,000	14,600	53	47	0
Second week	75	4,030,000	15,200	64	36	0
Third week	74	4,260,000	16,000	59	40	1
Fourth week	74	4,330,000	14,600	52	46	2
Fifth week	75	4,830,000	16,400	55	42	3
Sixth week	75	4,940,000	15,400	66	34	0
Average	74	4,533,000	15,270	58	41	1

GUINEA PIG 4—FEMALE, THIRTEEN WEEKS OLD

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES		
				P M N PER CENT	LARGE SMALL PER CENT	TRANS PER CENT
First week	74	4,380,000	14,800	64	36	0
Second week	74	4,200,000	15,400	61	36	0
Third week	75	4,650,000	14,800	67	32	1
Fourth week	74	4,200,000	14,000	56	41	3
Fifth week	75	4,770,000	15,000	69	31	0
Sixth week	73	4,540,000	14,400	59	39	2
Average	74	4,440,000	14,700	63	36	1

## SUMMARY

	HEMOGLOBIN PER CENT	TOTAL RED	TOTAL WHITE	LYMPHOCYTES		
				P M N PER CENT	LARGE SMALL PER CENT	TRANS PER CENT
Guinea Pig 1	79	5,093,000	14,200	57	41	2
Guinea Pig 2	77	5,200,000	14,100	63	35	1
Guinea Pig 3	74	4,533,000	15,270	58	41	1
Guinea Pig 4	74	4,440,000	14,700	63	36	1
Average	76	4,816,500	14,560	60	38	1

For the hemoglobin, the Sahli method was used. This consists in filling the estimation tube to the 10 mark with tenth-normal hydrochloric acid and carefully expelling the blood, which has been drawn up to the 20 mm mark in the hemoglobin pipette, below the surface of the acid. The blood is allowed to hemolyze for two minutes and then diluted with distilled water until the color matches the standard color rods. The percentage is then read directly. Two counts were made on all experiments, the results checked to within experimental error. White cells varied not more than 200 to 250, and red cells 50,000 to 100,000. A mean of the two counts was recorded.

A survey of the many blood counts above shows

- The total white counts of ages from thirteen weeks to thirty weeks average 14,560
- There are no constant differences in the total white counts of the guinea

pigs of ages from thirteen to thirty weeks or between males and females of these ages

c There is an average red cell count of 4,816,500 and the ranges are similar in both males and females of the ages studied

d The hemoglobin percentage averages 76

e The lymphocytes, large and small, were difficult to separate They are, therefore, grouped together

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# LABORATORY METHODS

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## THE COLORIMETRIC DETERMINATION OF PLASMA PROTEINS\*

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IF AN alkaline solution of the blood protein is heated for a short time, the chromogenic value, when treated with Folin-phenol reagent, is markedly intensified. Thus serum-globulin gives half again as much color when so treated and serum-albumin twice as much. A maximum chromogenic effect is produced by one-half hour heating in a water-bath, using 10 per cent sodium hydroxide as the protein solvent.

This procedure is introduced into the following adaptation of the colorimetric method of Wu,<sup>1</sup> Wu and Ling,<sup>2</sup> and the late modification of Greenberg.<sup>3</sup> Further simplifications of the technique of the colorimetric protein method have been introduced and a rapid and more accurate colorimetric procedure for the determination of blood proteins is offered.

Greenberg, in his modification, used sodium sulphate according to the Howe separation of protein.<sup>4</sup> The necessity of keeping the sodium sulphate at 37° to keep it in solution, and the difficulty of filtration, besides the long time necessary for the complete precipitation of the globulin, led us to discard the Howe procedure and use the precipitation with ammonium sulphate. Robertson<sup>5</sup> reviewed the methods for the separation of protein and concluded that the ammonium sulphate precipitation was the most satisfactory. Wu, and Wu and Ling, as well as Cullen and Van Slyke<sup>6</sup> also found this a satisfactory separation.

Wu had noted in his colorimetric determination of protein that if the protein precipitate was allowed to stand with sodium hydroxide for a short time, or with sodium carbonate for a long time, that the chromogenic value was increased, but he made no attempt to incorporate this into his method.

We found that by heating for a half hour, the chromogenic value not only is constant and maximum, but the tyrosine equivalents for the plasma proteins are roughly the same. This permits the determination of the total proteins of plasma and spinal fluid with sufficient clinical accuracy irrespective of the protein partition.

The development of color with the Folin-phenol reagent<sup>7</sup> was considered satisfactory. This reagent gives some precipitate, this, however, has no effect on the color, and settles so rapidly that it does not interfere with the colorimetric reading. We did not find that modified reagent by Folin and Ciocalteu<sup>8</sup> gives an appreci-

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ably deeper color with the alkaline protein than did the original reagent as stated by Greenberg

#### METHOD

*Procedure* —To 1 c c of plasma in a centrifuge tube, add 2 c c of water, and 1 c c of saturated ammonium sulphate (56 gm per 100 c c) Mix and centrifuge after a few minutes to separate the fibrinogen Pour off the supernatant fluid containing the albumin and globulin, and reserve the precipitate of fibrinogen

One cubic centimeter of the supernatant fluid is pipetted into a 15 c c centrifuge tube, and 0.5 c c of water and 1 c c of saturated ammonium sulphate added This brings the solution to half saturation Let stand for twelve hours at room temperature, or at 40° for fifteen minutes Centrifuge and pour off the supernatant fluid for the albumin determination Dissolve the globulin precipitate in 5 c c water

Pipette 2 c c of reserve fluid containing the albumin into a centrifuge tube, and add 3 c c of water Then add 1 c c of 20 per cent trichloroacetic acid to both the globulin and albumin solutions Centrifuge and pour off the supernatant liquid Dissolve the precipitates of albumin, globulin, and fibrinogen in 0.5 c c of 10 per cent sodium hydroxide and heat in boiling water-bath for one-half hour Add 7.5 c c water to all the tubes

To 2 c c of 20 mg per cent tyrosine standard solution (in N/10 HCl), in a centrifuge tube, add 6 c c water To each of the proteins and to the standard, add 1 c c Folin phenol reagent and 3 c c of saturated sodium carbonate and mix by inversion Read after thirty minutes in a colorimeter, setting the standard at 10 mm

*Calculation* —The tyrosine equivalents were found to be

$$\begin{aligned} 1 \text{ mg tyrosine} &= 12.9 \text{ mg albumin} \\ 1 \text{ mg tyrosine} &= 13.1 \text{ mg globulin} \\ 1 \text{ mg tyrosine} &= 13.0 \text{ mg fibrinogen} \end{aligned}$$

*Albumin* —

$$\begin{array}{r} 10 \times 0.4 \times 100 \times 12.9 = \text{mg albumin per 100 c c} \\ \hline R \quad 0.2 \\ \hline \text{or } 25.8 = \text{per cent albumin} \\ \hline R \end{array}$$

*Globulin* —

$$\begin{array}{r} 10 \times 0.4 \times 100 \times 13.1 = \text{mg globulin per 100 c c} \\ \hline R \quad 0.25 \\ \hline \text{or } 21.0 = \text{per cent globulin} \\ \hline R \end{array}$$

*Fibrinogen* —

$$\begin{array}{r} 10 \times 0.4 \times 100 \times 13.0 = \text{mg fibrinogen per 100 c c} \\ \hline R \quad 1.0 \\ \hline \text{or } 5.2 = \text{per cent fibrinogen} \\ \hline R \end{array}$$

#### TOTAL PLASMA PROTEIN

Due to the close agreement of the tyrosine equivalents, it is possible to determine total protein by using 13 as the tyrosine equivalent

#### METHOD

*Procedure* —Plasma is diluted 1:10 with saline and 1 c c is transferred to a 15 c c centrifuge tube and 4 c c of water and 1 c c of trichloroacetic acid are added The precipitate is centrifuged out and the supernatant fluid poured off The precipitate is dissolved in 0.5 c c of 10 per

cent sodium hydroxide and heated in a water bath for a half hour. The color is then developed as given previously for the fractions.

*Standard*—The same standard is used as is used for the fractions.

*Calculation*—

$$\frac{10 \times 0.4 \times 100 \times 13.0}{R \quad 0.1} = \text{mg protein per 100 c c}$$

$$\text{or } \frac{52.0}{R} = \text{per cent protein}$$

#### PROTEIN IN SPINAL FLUID

##### METHOD

*Procedure*—Two cubic centimeters of spinal fluid is measured into a 15 c c centrifuge tube and 3 c c water and 1 c c 20 per cent trichloroacetic acid is added. The protein precipitate is centrifuged out and the supernatant fluid is poured off. The precipitate is dissolved in 0.25 c c sodium hydroxide and heated one half hour in a water bath. Then add 3.75 c c water, 0.5 c c phenol reagent, and 1.5 c c saturated sodium carbonate. Mix by inversion and read in a half hour, setting the standard at 10 mm.

*Standard*—Two standards should be made up, one containing 1 c c 20 mg per cent tyrosine and 7 c c water, and the other 2 c c standard and 6 c c water. Then add 1 c c phenol reagent and 3 c c saturated sodium carbonate to each tube.

*Calculation*—Standard 1

$$\frac{10 \times 0.2 \times 100 \times 13.0}{R \quad 2.0 \quad 2.0} = \text{mg protein per 100 c c}$$

$$\text{or } \frac{650}{R} = \text{mg protein}$$

Standard 2

$$\frac{10 \times 0.4 \times 100 \times 13.0}{R \quad 2.0 \quad 2.0} = \text{mg protein per 100 c c}$$

$$\text{or } \frac{1300}{R} = \text{mg protein}$$

##### EXPERIMENTAL

In order to determine the tyrosine equivalent of fibrin, 400 mg fibrin (Kahlbaum) was heated for forty minutes with 50 c c of 10 per cent sodium hydroxide and 25 c c of water. The solution was made up to 100 c c and 1 c c taken for the determination. The tyrosine equivalent under the conditions of the method was 12.9.

This value was checked by a nitrogen determination. A macro-Kjeldahl was run on ovalated plasma. To another portion of the plasma a trace of calcium chloride was added, and after twenty minutes, the clot was broken up, and a Kjeldahl run on the serum. The difference in nitrogen values multiplied by 5.91<sup>10</sup> gave the amount of fibrinogen. The equivalent was 13.

The tyrosine equivalents of the albumin and globulin were determined by comparison of the colorimetric determination with the protein values based on nitrogen content of the fractions. Nonprotein nitrogen of the serum specimen was determined according to Folin and Wu.<sup>11</sup> The globulin was precipitated by the method of Howe,<sup>4</sup> and Kjeldahl. The nonprotein nitrogen was subtracted from

the total nitrogen of the filtrate from the globulin precipitate. This value and that of the globulin nitrogen were multiplied by 6.29<sup>1</sup> to obtain the amount of these proteins.

A series of determinations was run on normal and patients' serums, and

TABLE I

A COMPARISON OF THE PLASMA PROTEIN VALUES OBTAINED BY THE COLORIMETRIC METHOD AND BY DETERMINATION BASED ON THE NITROGEN CONTENT

SPECIMEN	FRACTION	PER CENT PROTEIN		DEVIATION
		COLORIMETRIC	NITROGEN	
Mixed plasma	albumin	3.38	3.54	-0.16
	globulin	2.64	2.62	+0.02
	total	6.02	6.16	-0.14
Mixed plasma	albumin	3.85	3.75	+0.10
	globulin	2.68	2.62	+0.06
	total	6.53	6.37	+0.16
Nephrosis	albumin	1.99	2.10	-0.11
	globulin	2.40	2.39	+0.01
	total	4.39	4.49	-0.10
Cardiac with edema	albumin	2.90	3.03	-0.13
	globulin	1.45	1.43	+0.02
	total	4.35	4.46	-0.11
Nephrosis	albumin	2.66	2.60	+0.06
	globulin	2.17	2.24	-0.07
	total	4.83	4.84	-0.01
Plasma saline	albumin	2.82	2.87	-0.05
	globulin	2.06	2.18	-0.12
	total	4.88	5.02	-0.17
Plasma saline	albumin	2.49	2.65	-0.16
	globulin	2.13	1.96	+0.17
	total	4.62	4.61	+0.01
Cardiac with edema	albumin	2.77	2.56	+0.21
	globulin	2.17	2.15	+0.02
	total	4.94	4.71	+0.23
Plasma saline	albumin	1.79	1.77	+0.02
	globulin	1.53	1.31	+0.22
	total	3.32	3.08	+0.24
Nephrosis	albumin	2.37	2.29	+0.08
	globulin	1.83	1.73	+0.10
	total	4.20	4.02	+0.18
Mixed plasma	fibrinogen	0.41	0.45	-0.04
Mixed plasma	fibrinogen	0.67	0.65	+0.02
Average Deviation				-0.02

on a few dilutions of these and the average equivalent determined. The average equivalent for albumin was 12.9 and for globulin 13.1.

Table I shows the comparison between the colorimetric method based on the tyrosine equivalents just given, and the protein based on nitrogen determinations.

#### SUMMARY

A modification and simplification of the Wu colorimetric method for the determination of plasma proteins is described. It has been found that not only a maximum chromogenic value but approximately equal tyrosine equivalents for the plasma proteins are obtained by heating these for a half hour with 10 per cent sodium hydroxide.

On the basis of these similar tyrosine equivalents, modifications are presented for the determination of total protein of plasma and spinal fluid which give values of sufficient clinical accuracy, irrespective of the protein partition.

A table gives a comparison of protein values obtained by this method and values based on nitrogen content.

#### ADDENDUM

Occasionally we have had plasma specimens from which the globulin failed to centrifuge out satisfactorily. In practice, we now determine the total protein on 0.1 c.c. of plasma heated a half hour with 0.5 c.c. of 10 per cent sodium hydroxide solution. The fibrinogen is separated as outlined and the supernatant fluid is drawn off completely, 2 c.c. of water and 4 c.c. of saturated ammonium sulphate are added and the mixture warmed at 37° C. for fifteen minutes. The globulin precipitate is removed by filtration through a pleated filter, the filtrate being returned onto the paper until clear. Albumin is determined on 2 c.c. of the filtrate. The globulin is figured by subtracting the sum of the fibrinogen and albumin from the total protein.

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## STUDIES IN THE SEROLOGY OF SYPHILIS\*

### XII MODIFICATIONS IN THE TECHNIC AND INTERPRETATION OF THE WASSERMANN REACTION

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DESPITE the general adoption of various precipitation tests, the Wassermann reaction remains the one test most widely used in the laboratory diagnosis of syphilis. Nevertheless, there is widespread disagreement as to the optimum technical procedure. Changes which have been suggested by various serologists from time to time have not been universally adopted, with the result that the technic employed in different laboratories may vary considerably, and with it, of course, the sensitivity and specificity of the results obtained. Moreover, certain points of view as to the interpretation of results have become so firmly entrenched by constant reiteration in the enormous literature as to appear almost axiomatic to the present day clinician and serologist, despite considerable evidence that some of these may be erroneous.

The present paper represents an attempt to determine, on the basis of the results obtained in this laboratory, the optimum adjustment of some of the factors on which serologists disagree, the temperature and duration of incubation, the number of antigens to be used, the proper degree of antigen sensitization, and the methods to be used in the standardization of the reagents. There are included some observations on the interpretation of Wassermann results, specifically, (1) the questionable value of 1+, 2+, 3+, and 4- reports as a quantitative scale of positivity, (2) the incidence of false positive Wassermann reactions in diseases other than syphilis, and (3) the nature of the active substance in syphilitic serum, so-called reagin.

#### A UNNECESSARY MULTIPLICATION OF ANTIGENS

Many laboratories test each serum with two, three, or even four different antigens. Two advantages might conceivably attach to such a procedure, but as will be shown, neither is realized in practice. Such reports as

Wassermann Reaction  
Plain antigen = negative  
Cholesterolized antigen = positive  
Acetone-insoluble antigen = doubtful

serve only to confuse the clinician, and destroy confidence in the particular laboratory.

In the first place, the use of multiple antigens does not serve as a check against false positive reactions. Given a serum which is positive with one antigen, and negative with another, this usually means only that the two antigens differ in sensi-

\*From the Syphilis Division of the Department of Medicine, Johns Hopkins Medical School. Received for publication June 17, 1932.

tivity, that is the serum does contain syphilitic reagin, but the amount of reagin in the particular serum is less than the threshold value for the weaker antigen. The incidence of biologic false positive Wassermann reactions, even with the most sensitive antigen available is so small that a persistently positive reaction may be taken as *prima facie* evidence of the presence of syphilitic infection (see Section C). It might be said that the use of multiple antigens is a check against such laboratory errors as failure to add complement, the use of an anticomplementary antigen, etc. No well-run laboratory uses antigen in more than a small fraction ( $\frac{1}{4}$ - $\frac{1}{8}$ ) of its anticomplementary dilution, while the best method of controlling the other types of error is to use several dilutions of serum. This procedure serves not merely as a mutual check, but as a semiquantitative titration of the reagin content of the serum.

On the other hand, one might argue that the use of several antigens makes doubly certain the detection of a syphilitic serum. It is true that if one tests a large number of syphilitic sera with several antigens, there will be a considerable difference in the number detected by the individual antigens (Table I). Nevertheless, as is seen in Table I, such differences are not haphazard, but depend solely upon the absolute sensitivity of the antigen. We find no evidence that antigens differ in their selective affinity for particular clinical conditions.

To demonstrate these points, the sera of 258 syphilitic patients undergoing active treatment for syphilis were examined with five different antigens. A second group of 265 similar cases were tested by the antigen used in this laboratory (Protocol 1 (e)) and by the Kolmer antigen supplied by the Digestive Ferments Co. The preparation of the antigens, and the technique of the test, are described in Protocol 1. The results are listed in Table I. As is there shown, the most sensitive antigen detects *every* serum picked out by the weaker antigens, *plus* a certain number which they missed because of their lower sensitivity. In not a single case did a "weak" antigen pick up a serum missed by the usually more sensitive antigen. It is obvious that no virtue attaches to the use of 2, 3, or 4 antigens. One need only select the most sensitive of the lot, and use it alone.

#### PROTOCOL I

*Preparation of Antigens*—a Fifty grams of dried powdered beef heart (Difco) were extracted with 250 c.c. of ether for fifteen minutes at 37° C. The ether filtrate was discarded and the moist powder was washed on the filter with 100 c.c. of warm ether, dried, and extracted at 37° C. for four days with 250 c.c. of 95 per cent ethyl alcohol. After filtration, the moist powder was washed with 100 c.c. of alcohol, and the combined alcoholic filtrate and washings evaporated down to 75 c.c. Seventy-five c.c. of absolute alcohol were then added.

b The extract obtained as in (a) was sensitized with 0.2 per cent cholesterol.

c Fifty grams of dried powdered beef heart (Difco) were extracted with 250 c.c. of alcohol for four days at 37° C. The alcoholic filtrate was evaporated down to dryness, and the soft residue washed 3 times with 100 c.c. of hot acetone. The acetone extracts were discarded, and the hard waxy residue dissolved in 5 to 10 c.c. of ether. Then 200 c.c. of hot 95 per cent alcohol were added, the mixture evaporated on the steam bath down to 150 c.c., and filtered. Cholesterol was added to a concentration of 0.4 per cent.

d The extract obtained as in (a) was sensitized with 0.6 per cent.

e The extract obtained as in (a) was sensitized with 0.8 per cent cholesterol and 0.6 per cent sitosterol, dissolved by boiling. This antigen (e) is the one in routine use at the Johns Hopkins Hospital. It has since been found that the antigen containing 0.6 per cent cholesterol and 0.6 per cent corn germ sterol yields identical results, with the added advantage that it can be

used in a flocculation test as well (Paper VIII of this series, J LAB & CLIN MED 17 787, 1932) Both antigens can be obtained from the Digestive Ferments Co., Detroit

f Kolmer antigen supplied by the Digestive Ferments Co

*Technic of Wassermann Test Used in All the Experiments Included in This Paper*—The clear serum was inactivated at 56° C for twenty minutes, and 0.1, 0.1, and 0.05 c c respectively were added to three tubes. Complement was prepared by pooling the blood of at least 5 pigs, allowing it to clot overnight at ice box temperature, and centrifuging. Two tenths c c of a 1:12 dilution in 0.85 per cent NaCl were added to all three tubes.

Antigen was used in a 1:200 dilution, prepared by dropping one volume of antigen slowly with shaking, into 200 volumes of salt solution\*. In the case of antigen e, the excess of sensitizer is redissolved by heating the antigen at 56° for a few minutes before diluting. Two tenths cubic centimeter of the dilution are added to Tubes 2 and 3, Tube 1 serving as an anticomplementary control.

Two tenths cubic centimeter of salt solution are added to Tube 1, making a total volume of 0.5, 0.5, and 0.45 c c respectively in the three tubes.

The tests were then placed in the ice box for four hours, followed by one half hour incubation at 37° C. Four tenths cubic centimeter of a sensitized cell suspension were then added and the results read in fifteen to twenty minutes.

The sensitized cells were prepared by washing citrated sheep's blood with 10 volumes of salt, and diluting the measured cells with 32 volumes of salt solution. The minimal dilution of amboceptor which will cause the hemolysis of this suspension in one half hour as then determined (0.2 c c of amboceptor + 0.2 c c 3 per cent cells + 0.2 c c 1:12 complement + 0.4 c c salt solution). The 3 per cent cell suspension was then sensitized with an equal volume of amboceptor dilution containing 2½ to 3 times this minimal hemolytic quantity, and 0.4 c c of the resultant 1½ per cent cell suspension used in the test.

#### B THE SUPPOSED DANGER OF FALSE POSITIVE REACTIONS WITH HIGHLY SENSITIZED ANTIGENS

The use of cholesterol to increase the sensitivity of Wassermann antigens is a generally employed procedure. Nevertheless, serologists have been unaccountably cautious in taking full advantage of its sensitizing properties, the majority of laboratories using it in a concentration of only 0.2 per cent. Yet, as may be seen from Table I and Fig 1, supersaturation with cholesterol, up to a concentration of 0.8 per cent, does not exhaust the possibilities of sensitization. A plain alcoholic antigen yielded 34 per cent of positive Wassermann reactions among a known syphilitic population who were under active treatment for syphilis, the same antigen containing 0.2 per cent cholesterol yielded 40 per cent, with 0.6 per cent cholesterol, 57 per cent, and with 0.8 per cent cholesterol plus 0.6 per cent sitosterol in addition, 70 per cent of positive tests among the same patients. In a general hospital population, excluding those under specific treatment for syphilis, the last named antigen detected 60 to 65 per cent more syphilitic sera than the same antigen without sensitizer (Table II).

The failure of serologists to take advantage of maximum sensitization as an aid to diagnosis is due to a widespread belief that highly cholesterolized antigens tend to yield false positive reactions. This impression I do not believe to be justified. It is true that a highly sensitized antigen is more anticomplementary than the plain alcoholic extract. Nevertheless, if due allowance is made for this increased anticomplementary activity by using the antigen in a small fraction of its anticomplementary concentration (less than 1/8), the following results indicate

\* See footnote on page 829. A more sensitive antigen dilution can be obtained by reversing the order of dilution: dropping the salt solution slowly with shaking into the antigen.

that an antigen containing even 1 4 per cent of sensitizing sterols (Protocol 1 (e) ) does not yield a significant proportion of false positive reactions, and that as already stated, a persistently positive Wassermann reaction with such an antigen is to be taken as prima facie evidence of syphilitic infection

TABLE I A

SHOWING THAT THE MORE SENSITIVE ANTIGEN WILL ALMOST INVARIABLY DETECT THOSE SERA PICKED UP BY WEAKER ANTIGENS I E., ANTIGENS DO NOT HAVE HAPHAZARD SELECTIVITY FOR PARTICULAR SERA OR FOR PARTICULAR CLINICAL CONDITIONS  
WASSERMANN RESULT WITH

NO OF SERA	ELAIN ALCOHOLIC EXTRACT		A 1 0 2 % CHOLESTEROL		ACETONE INSOLU BLE + 0 4 % CHOLESTEROL		A + 0 6 % CHOLESTEROL		A + 0 8 % CHO LESTEROL + 0 6 % SITOSTEROL		EAGLE FLOCCU LATION TEST <sup>1</sup>	
61	0		0		0		0		0		0	
17	0		0		0		0		0		+	
11	0		0		0		0		+		+	
2	0		0		0		0		+		0	
3	0		0		0		0		+		0	
1	0		0		0		0		+		+	
17	0		0		0		0		+		+	
18	0		0		0		+		+		+	
13	0		0		0		+		+		+	
2	0		0		0		+		+		+	
10	0		0		+		+		+		+	
6	0		+		+		+		+		+	
4	0		+		+		+		+		+	
5	0		+		+		+		+		+	
5	+		+		+		+		+		+	
1	+		+		+		+		+		0	
14	+		+		+		+		+		+	
68	+		+		+		+		+		+	
<hr/>												
Total	+	±	+	±	+	±	+	±	-	±	+	±
258	68	20	88	15	97	16	128	18	167	13	188	3
	26%	8%	34%	6%	37%	6%	50%	7%	65%	5%	73%	1%

TABLE I B  
WASSERMANN RESULT WITH

NO OF SERA	KOLMER ANTIGEN		ANTIGEN OF PROTOCOL 1 (E)	
88	0		0	
28	0		±	
41	0		+	
53	±		+	
55	+		+	
<hr/>				
265	+	±	+	±
	55	53	147	25
	28%	20%	55%	10%
		48%	66%	



1 Of 189 applicants for the blood transfusion donor list tested by such an antigen using the technic outlined in Protocol 1, only one gave the slightest trace of fixation that one case was found to have a darkfield positive chancre

2 Nine hundred and forty-six white, comparatively well-to-do patients in the private wards and in a semi-private outpatient department, in whom the incidence of syphilis is lower than in any other hospital group, were also tested by this

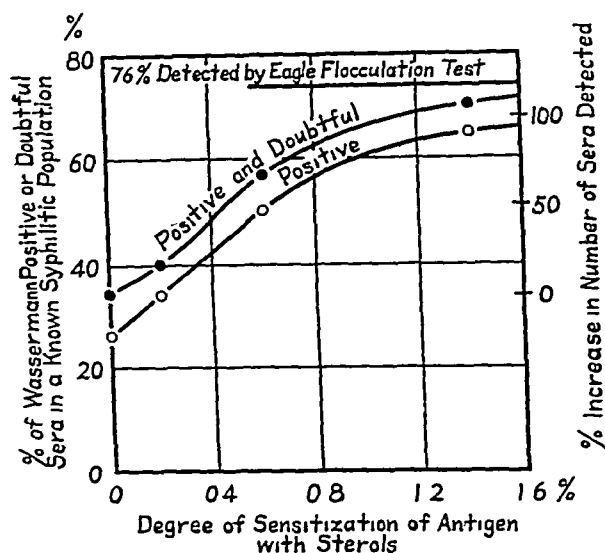


Fig 1—Showing the effect of increasing sensitization upon the sensitivity of a Wassermann antigen

strongly sensitized antigen The results are outlined in Table III There were 19 positive and 7 doubtful Wassermann reactions in the entire group

The doubtful reactions are omitted from consideration, as they include all tests with incomplete hemolysis, whether that was due to technical error or actual

TABLE II

SHOWING THAT A STRONGLY SENSITIZED ANTIGEN DETECTS 60 PER CENT MORE WASSERMANN POSITIVE SERA IN A GENERAL HOSPITAL POPULATION THAN A PLAIN UNSENSITIZED EXTRACT WASSERMANN RESULT WITH

NUMBER OF CASES		PLAIN ANTIGEN		EXTRACT SUPERSATURATED WITH STEROLS		
1039		0		0		
18		0		±		
30		0		+		
2		±		±		
15		±		+		
76		+		+		
Total 1180		76	17	0	121	32
		65%	15%	103%	27%	87%
		8%		12.9%		

fixation. Such reports are always accompanied with the request that the test be repeated. Of the positive tests, two were obviously technical errors, due to some one of many possible factors (inadvertent omission of complement, mislabeled specimens dirty tube etc.), for a simultaneous precipitation test and all subsequent Wassermann reactions were entirely negative. Such technical false positive (or doubtful) reactions can not be properly considered due to the nonspecificity of the antigen, for they are encountered with varying frequency in any large laboratory, no matter what the technic or antigen used, and are clearly not due to the antigen as such. Of the remaining 17 positive reactions, 8 were in cases with definite clinical manifestations of syphilis. In 4, there was strong presumptive evidence of syphilitic infection. In one case the test was not repeated. There remained only 4 cases in this entire series of 946 in which there was a persistently positive Wassermann reaction in the absence of any clinical findings or history suggestive of syphilis. In view of the fact that the serologic finding is the only evidence of infection in fully one-third of all Wassermann posi-

TABLE III  
SHOWING THAT AN ANTIGEN CONTAINING AS HIGH AS 1:4 PER CENT SENSITIZING STEROLS DOES NOT YIELD PERSISTENT POSITIVE REACTIONS IN NONSYPHILITIC INDIVIDUALS BASED ON 946 PRIVATE PATIENTS

WASSERMANN NEGATIVE	WASSERMANN POSITIVE—19 CASES				
	TECHNICAL ERROR	CLINICAL EVIDENCE OF SYPHILIS IN 8 CASES	SYPHILIS PROBABLE IN 4	NOT REPEATED	PERSISTENTLY POSITIVE IN ABSENCE OF HISTORY OR CLINICAL SIGNS
920	2	6 neurosyphilis 1 aortic insufficiency 1 optic atrophy	2 History of penile lesion and treatment 1 Probable aortitis 1 Husband had tabes	1	4

tive cases in our dispensary,<sup>2</sup> it is quite probable that these were similar cases of latent syphilis. Indeed, of 50 positive Wassermann reactions obtained with a plain nonsensitized extract on patients in the general dispensary, almost exactly the same proportion (30 per cent) were in patients who gave no history or clinical evidence of syphilis. Moreover, the 4 cases in question all gave a persistent and strongly positive precipitation test. It would therefore appear that sensitization of an antigen, per se, does not cause nonsyphilitic patients to give a positive Wassermann reaction in more than a very small and indeterminate fraction of one per cent of the cases.

The cases in the general hospital and dispensary population which yielded a negative or doubtful Wassermann reaction when tested by a plain extract, and a persistently positive Wassermann when tested by a strongly sensitized antigen (3.8 per cent of the total group), thus can not be interpreted as nonspecific false positive reactions due to excessive sensitization, but must be considered as syphilitic. The surprising number of such disagreements (3.8 per cent of a general hospital and dispensary population and 30 to 35 per cent of the total positives encountered) simply reflects the fact that the reagin titer of syphilitics varies between

wide limits (Fig 2) If one titrates a group of syphilitic sera one finds that a few contain 100, 200, or even 400 units of reagin Such sera will be Wassermann positive with almost any antigen A larger number, containing intermediate quantities (20, 30, or 50 units) will be Wassermann positive with moderately sensitive antigens, but may yield negative or doubtful results with plain extracts There remain a large proportion of known syphilitic sera, with comparatively small quantities of reagin, 0 to 10 units, which are usually missed by any save a highly sensitive antigen, and even then, may yield inconsistent results upon repeated tests

As is shown in Fig 2, the same variation in reagin titer is encountered in a group of Wassermann positive patients who had not received previous treatment for syphilis A considerable proportion of these (20 to 40 per cent) would be missed by weakly sensitized antigens We have already shown that maximal sen-

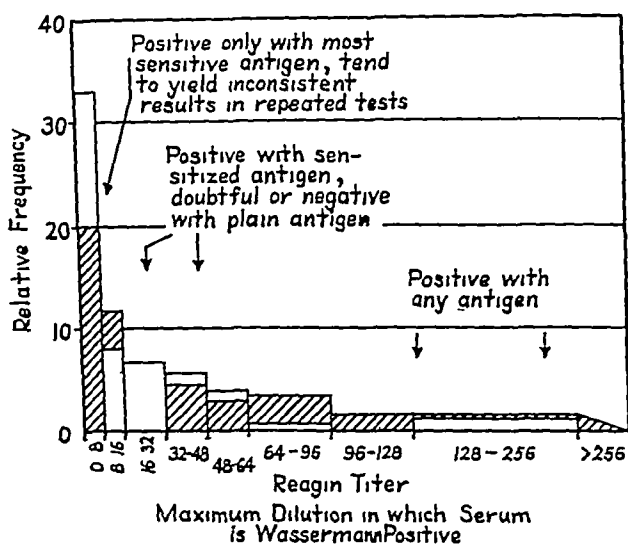


Fig 2—Variations in reagin titer among Wassermann positive sera. White area indicates known syphilitics under active treatment (146 cases). Oblique lines indicate previously untreated (120 cases).

sitization, even up to 1.4 per cent, while bringing about an increased sensitivity, does not predispose to false positive reactions to any significant extent, and there is thus no reason for the continued use of plain alcoholic extracts as Wassermann antigens

### C THE INADEQUACY OF THE WATER-BATH WASSERMANN REACTION

It is now twenty-two years since Jacobsthal<sup>2</sup> suggested that the sensitivity of the Wassermann reaction could be increased by incubating the mixture of complement, antigen, and serum at ice-box temperature prior to the addition of cells. As I have shown elsewhere,<sup>4</sup> this increased sensitivity depends upon at least four factors: (1) the prolonged incubation period, making for greater fixation of complement, (2) a spontaneous deterioration of complement, (3) a less marked inhibition of fixation by serum protein at the lower temperature, (4) a greater non-specific destruction of complement by antigen.

The difference in sensitivity between a thirty minute water-bath test, and a

TABLE IV

THE COMPARATIVE SENSITIVITY OF THE ONE HALF HOUR WATER BATH AND FOUR HOUR ICE BOX TEST IN A KNOWN SYPHILITIC POPULATION UNDER ACTIVE ANTISYPHILITIC TREATMENT

PLAIN ALCOHOLIC EXTRACT						SENSITIZED ANTIGEN						
NO OF CASES	WASSERMANN RESULT BY					NO OF CASES	WASSERMANN RESULT BY					
	WATER BATH INCUBATION 1½ HOUR	4 HOUR ICE BOX + ½ HOUR WATER BATH					WATER BATH INCUBATION 1½ HOUR	4 HOUR ICE BOX + ½ HOUR WATER BATH				
170	0			0		78	0			0		
17	0			±		13	0			±		
3	±			±		0	±			±		
26	0			+		58	0			+		
11	±			+		21	±			+		
31	+			+		88	±			+		
<hr/>												
Total	+	±	0	+	±	0	+	±	0	+	±	0
	31	14	213	68	20	170	88	21	149	167	13	78
	12%	6%		26%	8%		34%	8%		65%	5%	
	18%			34%			42%			70%		

test with the same reagents, but in which the tube is placed for four hours in the ice box before the water-bath incubation, is truly surprising. Of 258 known syphilis only 109 were detected by a water-bath test with the most sensitive antigen available, while 180 were detected by the combined four hour ice box and one-half hour water-bath test.

In not a single instance did the water-bath test detect a serum missed by the ice box incubation. Despite the greater sensitivity of the ice box test, it has not been universally adopted. As recently as 1928, of the seven serologists who carried out Wassermann reactions in the competitive League of Nations Conference, only one did a preliminary ice box incubation, and then only for one hour. At the 1930 Conference, of 7 Wassermann techniques, 6 were water-bath tests, and one involved a brief incubation at room temperature. Moreover, many laboratories which have adopted some type of cold incubation procedure continue to carry out a duplicate water-bath test, despite the fact that nothing is gained thereby.

As in the case of sensitization, this cautiousness can be attributed to a widespread, but in my opinion, mistaken impression that the ice box test yields false positive reactions. An eighteen hour ice box test proved occasionally unsatisfactory in this laboratory not because of biologic false positive reactions, but because of spontaneous deterioration of complement under the conditions of the test. In my own experience, however, the four-hour ice box test followed by one-half hour at 37° C. is entirely reliable, and as is shown by the data of pages 824 to 826, does not yield biologic false positive reactions, at least, if it does, the incidence is certainly less than an insignificant fraction of one per cent.

D THE OPTIMUM DURATION OF INCUBATION

Granted that a preliminary ice box incubation makes for a very significant increase in the sensitivity of the Wassermann reaction, what should be its duration?

A number of serologists have recommended that the antigen-serum-complement mixture be incubated eighteen hours in the ice box in order to obtain the maximum degree of complement fixation.<sup>5</sup> They consider that the increased sensitivity thus attained more than compensates for the inconvenience of a two day reaction. In this laboratory, however, such an overnight test has proved both unnecessary and undesirable, for the reasons discussed below.

1 We have not been able to confirm the finding that overnight incubation is necessary for maximum sensitivity. It is true that if a group of sera from patients under active antisyphilitic treatment is tested with a plain alcoholic beef heart extract, by the technic described in Protocol 1, incubating duplicate series of tests for varying periods of time in the ice box, there is a progressive and striking increase in the degree of complement fixation and in the number of positive reactions as the incubation period is prolonged. Thus, among 135 patients tested by one-half hour incubation at 37° C, there was a total of 16 per cent positive and doubtful reactions, increased to 37.7 per cent by preliminary two hours' incubation at 0° to 6° C, to 47 per cent by preliminary four hours at 0° to 6° C, and to 62.2 per cent by overnight ice box incubation (Fig 3). Fully 24 per cent of the ultimately positive reactions would have been missed by a preliminary four hour ice box incubation. If, however, a duplicate series of tests is carried out at the same time, under the same conditions, using the same basic antigen supersensitized with sterols (Protocol 1 e\*), quite different results are obtained. Instead of a progressive increase in the number of positive reactions over the entire range of one-half to eighteen hours' incubation, there is a sharp increase in sensitivity within the first two to four hours, but *further incubation has very little effect on the number of sera detected*. The fixation of complement is so accelerated by the highly sensitized antigen that the great majority (97.4 per cent) of those sera which are positive or doubtful after eighteen hours in the ice box are already so after four hours in the ice box + one-half hour at 37° C (Fig 3).

It follows that *with a sufficiently sensitized and properly diluted antigen*, the difference in the sensitivity of the overnight as compared with the four hour ice box test is not sufficient to justify the inconvenience of the longer incubation, particularly in view of the undesirable features of the latter described in the following paragraphs.

2 Whatever the cause for the anticomplementary action of serum, whether bacterial contamination, chemical impurities introduced into the blood, or an intrinsic property of certain sera, destruction of complement is a process in time—the longer the incubation period, the more complement is destroyed. It follows that sera apparently free from such properties when tested by incubation at 37° C for one half hour, may appear definitely so if the water bath incubation is preceded by four hours' incubation at 0° to 6° C, more to the point, *the number of anticomplementary reactions in an overnight ice box test is necessarily higher than that observed after four hours*, all other factors remaining the same. By the technic used in this laboratory, employing 2½ units of complement, 1 per cent of the general

\*The antigen in this experiment was prepared by dropping two volumes of salt solution slowly and with shaking into 1 volume of antigen. After a two- to five-minute interval the remaining 1½ volumes of salt solution were added. Such a dilution is more turbid and more sensitive than that prepared by dropping the antigen into the salt solution. Otherwise the technic is as described in Protocol 1.

hospital dispensary population and 2.5 per cent of those receiving antisyphilitic therapy yield anticomplementary results when tested by a four hour ice box, one-half hour 37° test. With overnight ice box incubation, these are increased to 3.5 and 6.5 per cent respectively. Any attempt to overcome this tendency, as by increasing the amount of complement, tends to defeat the very purpose of the overnight test, by making the reaction less sensitive.

3. Although the hemolytic activity of fresh complement from healthy guinea pigs remains practically unchanged after overnight incubation as a 1:30-1:60

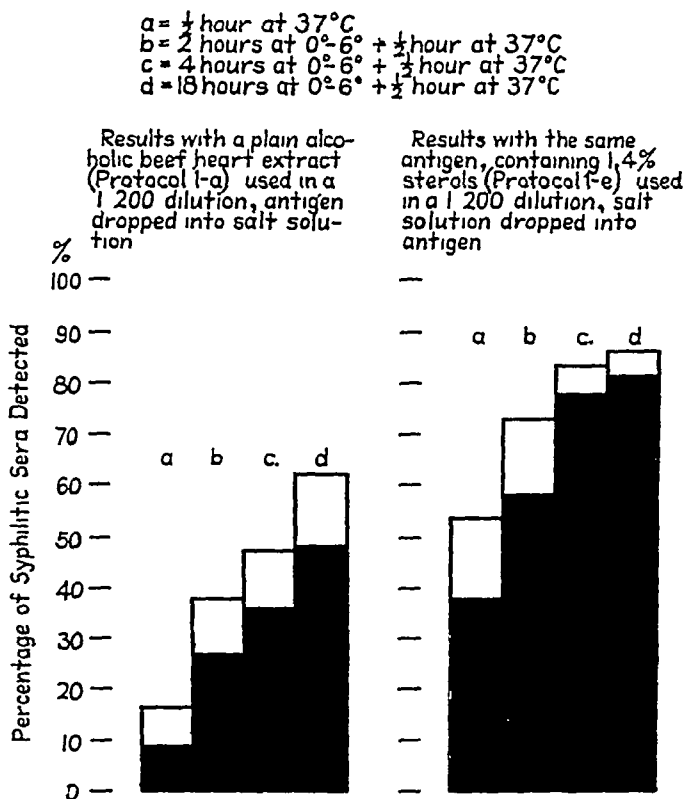


Fig. 3—Showing that with a highly sensitized antigen the overnight ice box test is not appreciably more sensitive than the four hour ice box plus one-half hour water-bath test. Solid black indicates positive reaction (no hemolysis). White area indicates doubtful reaction (partial hemolysis).

dilution, particularly in the presence of serum, complement from sick, anemic, or poorly nourished pigs may, in our experience, occasionally deteriorate so markedly during the overnight incubation as to invalidate the results of an entire series.

4. A strongly sensitized antigen is so anticomplementary upon overnight incubation that a quantitative titration of the serum is usually impossible. The qualitative test with whole serum, or serum diluted with two, three, or four volumes of salt solution may be quite satisfactory, inasmuch as the serum masks the anticomplementary action of the antigen. If, however, the test is performed upon serial dilutions of serum in order to determine its reagin content quantitatively, the antigen is usually so anticomplementary as to preclude the reading of the test.

in the higher serum dilutions. The use of weaker, that is, less strongly sensitized antigens, destroys the very purpose of the overnight incubation.

For these reasons, we have adopted as the routine in this laboratory a four hour ice box incubation, followed by one-half hour at 37° C *before* the addition of sensitized cells, using an antigen containing 1 1/2 per cent sterols and diluted with 200 volumes of salt solution in such a manner as to ensure maximum sensitivity (see footnote p. 829).

#### E. THE "TITRATION" AND "STANDARDIZATION" OF WASSERMANN REAGENTS

Every reagent used in the Wassermann reaction affects the sensitivity and specificity of the results obtained, as a result, the most important duty of the Wassermann technician is to make the necessary adjustments in the reagents. The antigen lipoid must be sufficiently concentrated and sensitized to ensure the detection of weakly positive sera, yet it must not be hemolytic or anticomplementary in the dilution used. The cell suspension must be sufficiently heavy to ensure a ready distinction between a positive (no hemolysis) and a negative result (hemolysis), the varying fragility of the cells must be properly compensated for. The complement and amboceptor activity must be controlled, as too much complement (or amboceptor) obscures weakly positive sera, while if there is too little complement or amboceptor, or if for some reason the complement deteriorates under the conditions of the test, the cells will fail to hemolyze in positive and negative sera alike. Moreover, the level of sensitivity must be kept fairly constant, else the results with sera of identical reagent content will vary from day to day.

To safeguard against any of these eventualities, there has been developed a maze of titrations and cross-titrations which constitute the technician's stock-in-trade. Without wishing in the slightest degree to detract from the necessity of rigid control, I do believe that these are unnecessarily complex, and that the technician can save himself considerable labor of reduplication by keeping in mind a few simple fundamentals.

1. *Antigen Titrations*—When antigens were prepared by simple alcoholic extraction of wet tissue, it was unavoidable that the alcohol concentration and lipoid content of final antigen were a matter of chance, and that the extract should be both anticomplementary and hemolytic. Of necessity such antigens had to be carefully titrated so as to be used in a dilution which, although possessing neither of these undesirable properties, should yet be sufficiently "antigenic" to detect positive sera. By modern methods of preparation, however, such repeated titrations become unnecessary. Antigens can be prepared, not occasionally, but uniformly which are not demonstrably hemolytic, are insignificantly anticomplementary, and yet are so active that their antigenic activity, after an initial test, need not be checked again. They can be used every day in the same dilution, with the assurance that one is using from 10 to 50 antigenic "units," and yet that one is 40 times removed from the danger zone of an anticomplementary antigen. A recommended method of preparing such an antigen is that described in Protocol 1 (e).

2. *Complement and Amboceptor Titrations*—Individual guinea pigs vary enormously in the complement (hemolytic) activity of their sera. The technician attempts to correct for this variation by determining the minimal hemolytic

quantity of complement, and by using some arbitrary but fixed number of such units in the actual reaction. Such a calibration ignores completely the fact that sheep cells vary by as much as 300 to 500 per cent in their susceptibility to hemolysis. Thus, comparing the bloods of the four sheep used in this laboratory, the most resistant requires  $3\frac{1}{2}$  times as much complement per unit cell volume as the least resistant, all other factors remaining constant. Obviously a calibration of complement in which cells are used as the yardstick, is, if not futile, quite inadequate. The hemolytic unit of complement may be 0.2 c.c. of a 1:30 dilution one day, and apparently the same two weeks later. Yet, because two different cell suspensions are used as the test object, the one unit may contain 2 to 3 times as much active complement as the other, with corresponding undesirable effects upon the sensitivity of the test.

There is another even more obvious error in the usual method employed to determine the complement "unit." Using a given cell suspension and a given quantity of complement, say 0.2 c.c. of a 1 to 10 dilution, the hemolytic quantity of amboceptor (unit) is first determined. An arbitrary multiple of amboceptor units, say three, are then added to the cells. With this sensitized cell suspension, the minimal hemolytic quantity of complement is then determined. This second titration of complement is meaningless, as it is predetermined by the number of amboceptor units used in the sensitization of the cells. If one unit of amboceptor had been used, the 0.2 c.c. of a 1:10 dilution of complement are barely hemolytic, and are thus one unit; with two units of amboceptor, the complement is found to be hemolytic in 1:20 dilution, and 1:10 complement will be two units; if 3 units of amboceptor are used, the complement may hemolyze the cells up to a 1:25 dilution, and 1:10 complement will be two and a half units. Clearly, this complement titration, upon which so much stress is laid as a means of standardizing the reaction, is in reality determined solely by the amount of amboceptor used to sensitize the cells.

The author believes the following method of calibration to be at least as reliable as any now in use, with the added advantage that it eliminates to a large extent the necessity for such complex cross titrations as just outlined.

a Use pooled complement of at least 3 or 4 pigs, and preferably more. Use it always after allowing it to clot for twenty-four hours in the ice box. In this manner, the variations between the activity of individual pigs can be minimized, and the complement taken as a more or less standard reagent.

b Use a constant quantity of pooled complement every day, e.g., 0.2 c.c. of a 1:10 dilution (+0.2 c.c. of antigen dilution, +0.2 c.c. of the serum dilution).

c Correct for the varying fragility of the cell suspensions by determining the minimal hemolytic unit of amboceptor each day, using the same amount of complement and cells in the titration as is to be used in the test. Sensitize the cell suspension with some arbitrary multiple of this unit, e.g., 3. With such a cell suspension, one will always be using 2 or 3 units of complement in the test—that is, the cell suspension is hemolyzed by 0.2 c.c. of 1:25 complement, as compared with the 1:10 complement used in the test. This provides an ample margin of safety for slightly anticomplementary sera, slight deterioration of complement, etc.

d Test for deterioration of complement under the conditions of the test by



determining the minimal hemolytic dilution before and after the proper period of incubation. Thus, for the technic outlined in Protocol 1 to 0.2 c.c. of 1:10, 1:20, 1:25, 1:30 and 1:40 complement add 0.4 c.c. of saline and incubate for four hours in the ice box and one half hour at 37° C. Add 0.4 c.c. of the cell suspension prepared as described in the preceding paragraph, and read hemolysis after one hour. If the readings differ appreciably from those obtained with freshly diluted complement there has been deterioration of complement. Slight deterioration in the 1:25 dilution or higher will have no effect in the test. Thus,

Complement dilution	1:10	1:20	1:25	1:30	1:40
Reading of hemolysis with freshly diluted complement	4	4	4	2	1
With complement diluted and incubated	4	4	2	2	0

implies minimal deterioration in the 1:10 dilution, and a satisfactory complement. On the other hand, a reading such as

	1:10	1:20	1:25	1:30	1:40
Freshly diluted complement	4	4	4	2	0
Complement after incubation	4	2	0	0	0

implies considerable deterioration of complement, and means (1) that the results in the particular series of tests must be read with the greatest caution, and (2) that the guinea pigs from which the complement was obtained must be given a rest or replaced.

#### F. THE SO CALLED 1+, 2+, 3+, AND 4+ (COMPLETELY POSITIVE) WASSERMANN REACTIONS

It has become traditional to report Wassermann results on a scale of plus marks. On this basis, 0 is a negative reaction, in which the complement remains free, and the cells hemolyze completely, 1+ is a "weak positive" reaction, in which the complement has been fixed to a slight extent, so that the tube remains hazy with undissolved cells, 2+ and 3+ represent increasing degrees of positivity, with more and more complement fixed, and decreasing degrees of hemolysis, 4+ is, on this scale, the strongest type of reaction, in which there is no detectable hemolysis, due to "complete" fixation of the complement.

TABLE V  
SERUM DILUTION

	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Results with 8	4+	4+	4+	4+	4+	4+	4+	4+
"completely positive"	4+	4+	2+	0	0	0	0	0
(1+) sera	4+	4+	4+	4+	4+	3+	0	0
	4+	4+	4+	4+	4+	0	0	0
	4+	4+	4+	4+	0	0	0	0
	4+	4+	4+	4+	4+	4+	4+	0
	4+	4+	0	0	0	0	0	0
	4+	4+	4+	4+	4+	1+	0	0

Actually, as Vedder<sup>6</sup> and Keidel<sup>7</sup> have shown, such a quantitation is entirely illusory, and does not even begin to reflect the total possible variation in the reagin content of syphilitic sera. If one selects a group of "4+" sera at random, and determines the maximum dilution in which this positive reaction can be obtained, one finds that there is a truly enormous variation, illustrated in Table V and Fig 4.

Some of the sera contain barely enough reagin to be positive with whole serum, some can be diluted 5 fold, while some continue to give a 4+ reaction even when diluted with 10, 40, or several hundred volumes of saline.

The degree of positivity of syphilitic sera can thus be expressed on a titer scale analogous to that of the Widal reaction (Fig 4). The term 4+ does not mean a

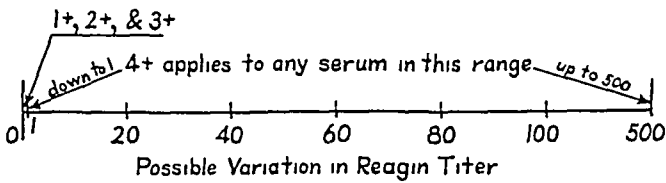


Fig 4—Showing the inadequacy of 1+ 2+ 3+ and 4+ reports as a quantitative scale of positivity

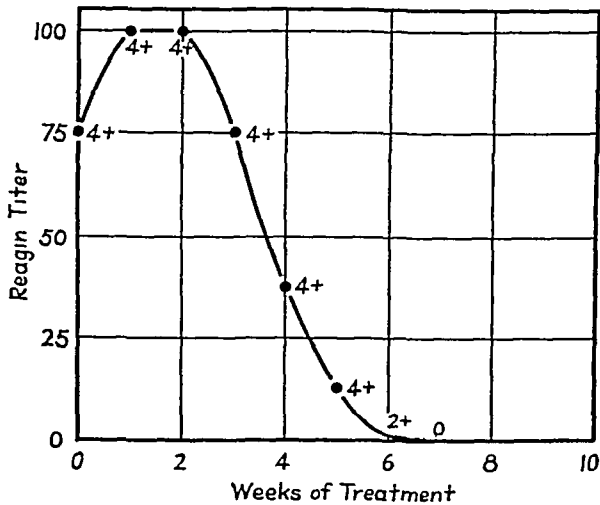


Fig 5—Serologic response of a patient with early syphilis to treatment with arsphenamine

strongly positive reaction, but applies to any serum which is Wassermann positive, whether that serum contains one or 400 units of reagin, while the terms 1+, 2+, 3+ upon which so much stress has been laid as a quantitative measure of positivity, actually cover a very minute and insignificant portion of the entire possible variation.

A frequent clinical observation becomes intelligible in the light of this quantitative aspect of the Wassermann reaction. A patient with early syphilis placed under specific therapy may continue to give a 4+ Wassermann for four, eight, twelve, or even sixteen weeks, and then quite suddenly, this "strong" Wassermann reaction breaks and becomes negative, frequently without passing through

the intermediate stages of 1, 2, or 3-. As a matter of fact, such a series of weekly Wassermann reactions (e g, 4, 4, 4, 4, 4, 4, 4, 4, 2, 0) gives not the slightest clue to the actual course of events. The patient's serum may have had an original titer of 200, i e, 200 times as much reagin as is necessary to give a 4+ reaction, under specific therapy, this may have fallen, week by week, to 100, 50, 25, 10, 5 with the routine reaction remaining 4+ all the while. It is only in the last part of the curve, when the serum reagin has almost completely disappeared, that the Wassermann breaks, and one obtains the long awaited serologic evidence of improvement in the appearance of 3, 2, 1+, or negative results (Fig 5)

It follows that

1 Reports should be made, not as 0, 1-, 2-, 3-, 4-, but, as the League of Nations Serological Conference suggested in 1928, as negative (complete hemolysis), positive (no hemolysis), or doubtful (partial hemolysis)

2 A doubtful report in a patient not known to have syphilis may mean either that the serum contains minute quantities of reagin, but not enough to prevent hemolysis entirely, or, that the reagents were not properly adjusted. It calls for careful serologic and clinical check-up

3 If a truly quantitative test of a positive serum is desired, it is necessary to determine the maximum dilution in which it continues to yield a positive result. The rate with which this Wassermann titer falls toward negative under treatment may be of distinct prognostic significance.<sup>8</sup>

4 If the patient's Wassermann fluctuates from week to week, as e g 0, 2-, 3-, 0, 4+, such variation is not necessarily a reflection on the laboratory, nor is it significant of any change in the patient. It may mean only that the serum in question contains minute quantities of reagin, comparatively slight but unavoidable variations in the delicacy and adjustment of the test would account entirely for the apparently discrepant results.

#### G THE SO CALLED BIOLOGIC FALSE POSITIVE WASSERMANN IN CONDITIONS OTHER THAN SYPHILIS

False positive reactions we have always with us. The great majority of such reactions are due to some technical error in the laboratory— inadvertent omission of complement, deterioration of complement under the conditions of the test, anti-complementarily antigen, mislabelled specimens, etc. Fortunately, such mistakes are usually readily detectable by repeating the test before instituting treatment. Of much greater theoretical interest is the other type of false positive reaction, the true fixation of complement supposed to be obtained in conditions other than syphilis. In the early papers on the subject, all sorts of conditions and diseases, including pregnancy, leprosy, tuberculosis, pneumonia, scarlet fever, etc, were reputed to predispose regularly to nonspecific reactions. Gradually it came to be recognized that the bulk of these were not biologic false positive reactions, but were due either to the actual presence of syphilis, or to technical errors. Nevertheless, despite numerous papers in the literature to the contrary, the impression persists that nonspecific positive reactions are obtained frequently even in temperate climates, where yaws, recurrent fever, and trypanosomiasis can be more or less definitely excluded.

TABLE VI

DISEASE	NO OF CASES	WASSERMANN NEGATIVE	ANALYSIS OF CASES WITH POSITIVE WASSERMANN			FALSE POSITIVE REACTION	DOUBTFUL WASSERMANN			FALSE DOUBTFUL REACTION
			TOTAL NO	SYPHILIS PROVED	SYPHILIS DOUBTFUL		TOTAL NO	SYPHILIS PROVED	SYPHILIS DOUBTFUL	
Pneumonia	87	73	12	8	3	1	2	2	0	0
Scarlet fever	14	14	0	0	0	0	0	0	0	0
Measles	13	13	0	0	0	0	0	0	0	0
Vincent's angina	5	3	1	1	0	0	1	1	0	0
Malaria	3	1	1	1	0	0	1	0	0	1
Acute infection	16	14	2	2	0	0	0	0	0	0
Unexplained fever	12	11	0	0	0	0	1	1	0	0
Tuberculosis	62	55	6	4	1	1	1	0	1	0
Alcoholism	38	34	3	1	2	0	1	0	1	0
Jaundice	62	49	11	9	2	0	2	0	1	1
Carcinoma pancreas	19	19	0	0	0	0	0	0	0	0
Carcinoma gastroin- testinal tract	19	18	1	1	0	0	0	0	0	0
Carcinoma (miscel- laneous)	15	13	2	2	0	0	0	0	0	0
Liver cirrhosis	13	11	2	2	0	0	0	0	0	0
Liver necrosis or atrophy	14	11	3	2	1	0	0	0	0	0
Pregnancy	38	35	2	1	1	0	1	0	1	0
Total	430	374	46	34	10	2	10	4	4	2

The statistics presented in Table VI give not the slightest evidence that such is the case. Four hundred and thirty patients were diagnosed as follows: scarlet fever (14), pneumonia (87), tuberculosis (62), jaundice (62), unexplained fever (12), acute infection (16), measles (13), carcinoma (53), alcoholism (38), Vincent's angina (5), liver affections (27), pregnancy (38), and malaria (3), all being conditions supposed to predispose to false positive reactions, had a Wassermann test during the course of the disease with an antigen containing at least 0.6 per cent sensitizing sterols. Of the total, 304 were four hour ice box tests, the remainder one-half hour water-bath tests. In the entire group there were 46 positive and 10 doubtful reactions. The difference between this incidence (10.7 per cent positive, 2.3 per cent doubtful) and the incidence of positive and doubtful reactions obtained in the general hospital and dispensary population (approximately 10 to 15 per cent) is hardly significant.

Moreover, of the 56 cases in which some type of positive result had been obtained, 38 presented either clinical or autopsy findings considered diagnostic of syphilis, a history of antecedent antisyphilitic treatment, or a positive serologic test before the onset of the disease under consideration. Of the remaining eighteen cases, there were 10 positive and 4 doubtful reactions in which the data available on the records were inadequate to allow any decision as to the presence or absence of syphilis. Inasmuch as about one-third of Wassermann-positive cases encountered in this dispensary present no evidence of syphilis, either clinical or in their history, (Turner 1930) it is probable that the majority, if not all, of these 12 individuals were similar cases of latent syphilis.

There remain only 2 positive and 2 doubtful reports in this entire group of 430 which can be properly termed false positive results. Of these, one positive reaction in a patient with pulmonary tuberculosis was obviously a technical error, and not due to the tuberculosis, for when the test was repeated, eight days later, it was entirely negative. The other positive result was in a case of pneumonia, Wassermann positive during the acute illness, and negative after the crisis. Of the two doubtful results, one was a case of malaria, and one a case of jaundice. Both were Wassermann doubtful during the acute illness, and negative thereafter. The rarity of these biologic false positive and doubtful results (one and two respectively in a total of 430 cases), in the very conditions which are supposed to predispose to their appearance raises serious doubt as to the existence of any causal relationship.

#### H THE NATURE OF THE WASSERMANN REAGIN

The discovery that an alcoholic extract of normal tissue could be used as antigen in the Wassermann reaction seemed to prove definitely that the active substance in syphilitic serum was not an antibody to *Sp. pallida*. It is now generally believed that the Wassermann reaction is an anomalous phenomenon bearing not slightest resemblance to such true antigen-antibody reactions as bacterial agglutination, protein precipitation, or specific complement fixation. Despite this general impression, there is an increasing body of evidence that reagin may, after all, be a specific antibody produced in response to an antigenic stimulus.

To enumerate these points briefly

1 The substance responsible for both the Wassermann and flocculation tests appears some time after the infection, the interval corresponding roughly to the time which elapses between the beginning of artificial immunization in the bacteria and the appearance of specific antibodies in the experimental animal. When the infected individual is treated with specific drugs, there is a fall in the Wassermann titer, corresponding to the disappearance of antibodies when injections of antigen (bacteria, red cells, protein) into the experimental animal are discontinued.

2 The physical and chemical properties of the active substance in syphilitic serum resemble those of true antibodies. Like them, it does not dialyze, is associated with the globulin fraction of serum, is only moderately affected by temperature less than 55° C, and is rapidly and irreversibly destroyed by heat > 70° C. Like antibodies also, Wassermann substance is destroyed by formalin.\*

The physicochemical processes underlying the Wassermann and precipitation tests for syphilis are identical with those which determine specific aggregation and complement fixation. I have presented evidence elsewhere<sup>9</sup> to show that the active substance in syphilitic serum is deposited as a film of denatured protein around the colloidal dispersed lipid particles in the antigen dilution. It is this film of reagin globulin which is believed to adsorb complement (Wassermann reaction) and to cause the cohesion of similarly coated particles in the various flocculation tests (Kahn, Sachs, Georgi, Miller, Eagle, etc.). An exactly similar process determines the aggregation of bacteria, red cells, or dissolved protein by a specific

\*Literature summarized in J. Exper. Med. 52: 717, 1930.

antibody,<sup>10</sup> as well as the adsorption (fixation) of complement by the sensitized antigen particles<sup>11</sup>

4 Finally, it has been possible to induce a positive Wassermann reaction of extremely high titer in rabbits as an antibody response to the injection of a lipid antigen<sup>12</sup>

Granted that these several lines of evidence do strongly suggest that reagin is an antibody, it must be admitted that there is the greatest doubt as to the nature of the antigenic stimulus which initiates its production. The obvious answer, that the antigen is the *Spirocheta pallida*, as originally suggested by Wassermann and recently again proposed by Klopstock,<sup>13</sup> seems to fall down on several grounds: (1) the fact that reagin combines with *normal* tissue lipoids, (2) the experimental finding that cultures of supposed *Sp. pallida* usually fail to induce a positive Wassermann reaction when injected into rabbits<sup>14</sup> even though specific antibodies against the organisms are produced in high titer, and (3) the observation that cultures of *Sp. pallida* are not agglutinated by syphilitic sera<sup>15</sup> and cannot be used as antigen in the Wassermann reaction<sup>16</sup>. On the other hand, the suggestion of Sachs, Klopstock and Weil<sup>17</sup> that reagin is an antibody to autogenous lipoids, liberated at foci of infection and tissue destruction, seems to lack conviction. It is difficult to understand why only syphilis should cause such an intense autoantibody response, while infections in which the tissue destruction is just as marked fail to produce even traces of Wassermann reagin.

It is the feeling of the author, without as yet any experimental evidence, that the first conjecture is correct, and that reagin may after all be not an anomalous "colloidal state" but a true antibody to the *Sp. pallida*. The fact that normal tissue lipoids react with this antibody does not disprove this surmise for *Sp. pallida* and mammalian tissue may well contain a common antigenic factor, exactly as guinea pig, kidneys, sheep red cells, and various bacteria contain a common lipid, the heterophile antigen of Forssmann.

It is true that spirochetes cultured in artificial media usually fail to induce the formation of reagin in the experimental animal, and fail to react with the reagin of syphilitic sera. Nevertheless, the nonpathogenicity of these cultures for rabbits raises grave doubts as to the validity of their identification as *Sp. pallida*. This nonpathogenicity, and the aforementioned antigenic properties of the cultures, suggest indeed that such cultures may be either saprophytic contaminants, or degraded forms from which no conclusions as to the antigenic properties of *Sp. pallida* *in vivo* can be properly drawn.

The final decision as to whether reagin is an antibody to *Sp. pallida* must await the cultivation of a strain indubitably pathogenic for rabbits.

#### SUMMARY AND CONCLUSIONS

1 Progressive increase in the amount of sensitizing sterol in a Wassermann antigen causes a progressive increase in sensitivity, and does not result in the appearance of biologic false positive reactions. A supersensitized antigen (1 to 1½ per cent sterols) detects fully 50 per cent more syphilitic sera than are detected by a plain alcoholic extract. There is thus no need for the continued use of antigens containing either no sensitizer, or only 0.1 to 0.3 per cent.

2 It is unnecessary to use more than one good antigen. Different antigens

do not have selective affinity for particular clinical conditions, the number of sera they detect is determined solely by their sensitivity to reagin. When it is known which is the most sensitive of two or three antigens, no virtue attaches to the use of more than the one with maximum sensitivity.

3 The water-bath test (one-half hour at 37° C) compares so unfavorably in sensitivity with an ice box test (four hours at 0° to 6° C followed by one-half hour at 37° C) as to make some type of cold incubation almost mandatory. Since the latter does not predispose to biologic false positive reactions, it is unnecessary duplication to perform both types of test.

4 *With a highly sensitized antigen*, the difference in sensitivity between an overnight ice box test, and a test involving four hours in the ice box, followed by one-half hour at 37° C, is too slight to justify the longer incubation as a routine procedure. This is particularly true in view of the greater number of anticomplementary serum reactions and the danger of complement deterioration in the eighteen hour test, and the fact that a highly sensitized antigen cannot be used in an overnight test because of its anticomplementary properties.

5 There is no evidence that acute infections, malignancy, tuberculosis, pregnancy, liver disease, or alcoholism regularly predispose to false positive Wassermann reactions.

6 It is unnecessary to carry out a cumbersome daily or weekly multiple titration of antigen (antigenicity, hemolytic properties, anticomplementary properties). Modern methods of preparation ensure uniform, highly sensitive antigens. One need only determine the anticomplementary titer of a particular lot of antigen, once and for all, and use it always in the same dilution, sufficiently removed from this anticomplementary zone to eliminate any possibility of error on that score.

7 The daily titration of complement is an illusory quantitation inasmuch as the cell suspension used as the measure of complement activity, is itself highly variable. It is suggested that the complement can be roughly standardized by using the pooled serum of 3 to 7 pigs, using it always twenty-four hours after bleeding, and in a constant arbitrary dilution of e. g., 1:12. One can then compensate for fluctuations in cell fragility by adjusting the amboceptor, using some arbitrary but fixed multiple of the minimal hemolytic quantity in the test.

8 The terms, 1+, 2+, 3+, and 4+ Wassermann reaction are misleading insofar as they cover only an insignificant fraction of the total variation in the reagin content of syphilitic sera. The term 4+ does not necessarily imply a "strongly" positive reaction, but applies to any serum with more than one unit of reagin, whether that serum contains 1 or 200 units. It is therefore recommended that Wassermann reactions be reported as negative (complete hemolysis), positive (no hemolysis), or doubtful (incomplete hemolysis). If a truly quantitative reaction is desired, it is necessary to carry out a test on serial dilutions of serum (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, etc.).

9 Contrary to the accepted view that the Wassermann reaction is an anomalous and unique biologic test, there is a considerable body of evidence that the intimate physicochemical processes involved in the reaction are exactly similar to those which determine specific antigen-antibody reactions. That syphilitic reagin may after all be an antibody to products of infection. The nature of the antigenic sub-

stance responsible for its appearance, whether *Sp pallida*, as originally believed by Wassermann, or autogenous lipoids, remains in doubt

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## A METHOD FOR STUDYING THE ACTIVITY OF INTACT SKELETAL MUSCLE\*

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THE method herein described was employed by Leese, Hines, and Jordan<sup>1</sup> in studying the influence of fasting upon the activity of skeletal muscle. We believe that it has several distinct advantages over the methods ordinarily employed in this type of work. By this method one is able to study the activity of individual muscles with a minimum of disturbance to the natural physiologic conditions of the muscle.

A survey of the experimental work of previous investigators showed that in general either one of two procedures have been employed for the study of activity of individual muscles. The earlier and more frequently used method was that of attaching the isolated muscle to some form of recording apparatus. Although a great amount of information concerning muscular activity has been compiled by means of this method, its limitations for the study of fatigue are well recognized. The absence of circulation and nervous contact is sufficient to greatly alter the physiologic behavior. Realizing these difficulties, numerous workers have devised methods whereby muscular response may be recorded under conditions permitting an intact circulation with or without complete nervous contact. Cannon and Nice,<sup>2</sup> Gruber,<sup>3, 4, 5, 6</sup> Gruber and Fellows,<sup>7</sup> Gruber and Krietschmer,<sup>8</sup> Miley,<sup>9</sup> Gans and Miley,<sup>10</sup> Gans and Hoskins,<sup>11</sup> and numerous other investigators have used the cut tendon method. This method involves the sectioning of the tendon of an intact muscle and the tying of the muscle by means of a string to the apparatus in order that the muscular response may be recorded. In some instances undue stretching was prevented and in others little attention was paid to it. Experiments with and without the cut tendon suggested to the authors that the technique of tendon cutting usually resulted in disturbances difficult to control even though efforts were made to maintain conditions as normal as possible.

In order to prepare fatigued skeletal muscle for glycogen analyses, a method was designed to study muscular fatigue under conditions more ideal due to the absence of any operative treatment on the muscle used or on its attachments. Those disturbances to circulation and nervous behavior caused by the stretching or manipulation of the muscle were prevented by retaining the natural attachments of the muscle. The after-loading problem was eliminated in the same manner.

*Procedure*—The animals used for these experiments were adult rats selected from the general laboratory stock. Sodium amytal in doses of 6 mg. per 100 gm. of body weight was administered by the intraperitoneal route. This amount was sufficient to produce a satisfactory degree of anesthesia. The extent of the superficial

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reflexes was used as a criterion of the depth of anesthesia. Additional amounts of anesthesia were injected from time to time, either by means of the automatic apparatus built for this purpose or by a Luer syringe.

Break induction shocks were delivered directly to the gastrocnemius group of one limb of the rat at the rate of 2 to 3 per second. The contractions of the gastrocnemius group were recorded on a slow-moving extension kymograph. The stimulation was continued until no appreciable amount of shortening could be detected. The animal was then removed from the apparatus and the muscles excised and weighed. In some instances the tendon achilles was cut and tied to the recording device. After-loading adjustments were carefully made. In other experiments the recording apparatus was attached to the toes and the tendon left intact. Corrections were made for the calibration of the spring and magnification due to leverage.

*Description of Apparatus*—The stimulating unit permitted the sending of break stimuli to the tissue in uniform manner for as many hours as desired. It needed no attention except an occasional brushing of the mercury with a damp camel's hair brush. The unit was operated by a quarter horse power Westinghouse motor governed by a rheostat. The gearing was so arranged that the motor revolved sixty times to one revolution of the drum carrying the stimulating contacts. Such an arrangement should minimize the effect of slight variations in the house current. By means of a rheostat, any desired rate of stimulation could be effected between one stimulus every two seconds to one hundred stimulations per second.

The recording unit consisted of a Harvard recording lever, an adjustable calibrated spring, and a kymograph. The resistance of the Harvard lever and two pulleys was added to the load. The kymograph moved from 18 to 24 inches per hour and permitted about 1000 contractions to be recorded in a space of from 6 to 8 cm. The paper was adjusted so it moved in a slight spiral fashion. Such an arrangement permitted freedom from handling for as long as 20 or more hours. The rat was fixed to the apparatus by means of a modified femur clamp adjusted to the tibia. A slit  $\frac{1}{2}$  of an inch in length was made in the skin over the tendon and over the gastrocnemius, in order that the glass-shielded electrodes could be more easily pushed in place.

Fig 1 indicates the mechanics of the stimulating unit. The contact points *A*, *B*, and *C*, which were soldered to the drum, made contact with the mercury cups on the board, *K*. The couplers *A*, *B*, and *C*, represent the means of current departure from contacts made by the contact points of like letter. Each contact point was made with an adjustable coupler, into which was fastened a steel needle of the best quality. Steel was used because of the relative absence of alloy formation with mercury. Contact point, *D*, was similar to the others and operated in the mercury cup, *L*. The explanation for the various electrical contacts will be given in the discussion of Fig 2.

The drum to which the contact points *A*, *B*, and *C*, were fastened had a diameter of 160 mm, and was propelled by a belt, *F*, which in turn operated on a pulley shaft having a diameter of 10 mm. The shaft was the axis of a 160 mm diameter pulley upon which was placed a second belt to the motor shaft, the diameter of which was 5 mm.

A trip key, *G*, was fastened to the drum in such a manner that it turned the cyclometer, *H*. By this arrangement the total number of stimulations was automatically recorded.

The rubber tubes, *E*, were inserted into the board, *K*, and passed to a bottle, *M*. They were for the purpose of permitting the collection of mercury which was frequently brushed from the mercury cups. The mercury was cleaned and used again.

Fig 2 indicates the arrangement of the mercury cups on board, *K*, of Fig 1. Drawing *X* represents the arrangement of the mercury cups. The cups in the outer rows had a diameter of 1 cm. Each cup passed completely through the board and

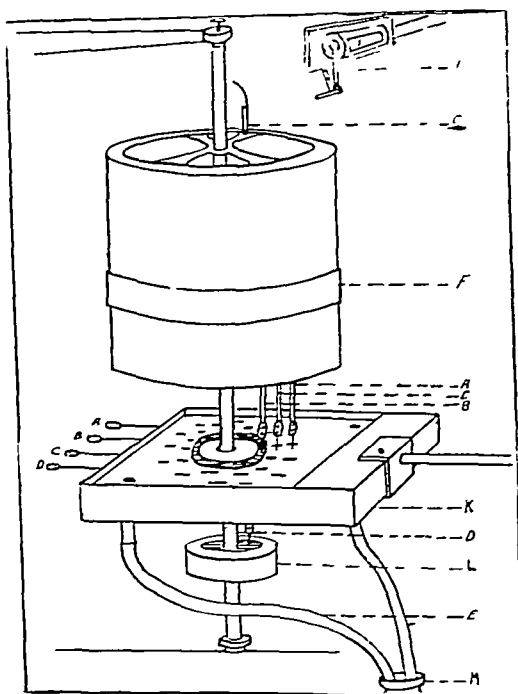


Fig 1—The stimulating unit. *A B C D* contacts and their electrodes. *E* rubber tubes. *F* belt. *G* cyclometer key. *H* cyclometer. *K* contact board. *L* mercury cup. *M* mercury bottle.

was plugged with a cork stopper containing a steel electrode as indicated in drawing *Y* of Fig 2. This arrangement made it possible to repair the connection to any cup if necessary, without any disturbance to the rest of the board.

Mercury cup, *C*, did not pass through the board, but was a moat 1 cm wide and 5 mm deep. It was also provided with a steel electrode from the under side of the board.

Drawing *Y* represents the opposite side of the board indicated in *X*, and presents the wiring of the mercury cups on board *K*.

It will be noted that lead *A* was a composite of the 16 outer cups which were in the primary circuit. Lead *B* was a composite of the inner row of 16 cups which were intermittent short circuiting keys in contact with one of the secondary poles of the inductorium. Lead *C* was in contact with the circular moat *C*, of diagram

$X$  and was a constant short circuiting key to the other pole of the secondary of the inductorium. By means of  $B$  and  $C$  the "make" current was automatically shorted out. The nature of the intermittent short circuiting keys,  $B$ , permitted the "short" to be broken in time to allow the "break" current to go through undisturbed.

The revolving drum,  $D$ , in diagram  $X$  shows the manner by which the shorting of the "make" was effected. Contact was made with  $B$  and  $C$  together before the primary current through  $A$  was closed. With the closing of  $A$  the "make" was shunted across the short circuit on the secondary and consequently did not reach the muscle. Contact with the intermittent short circuiting keys,  $B$ , was broken before  $A$  was broken. Therefore the break current went out over the secondary and to the muscle.

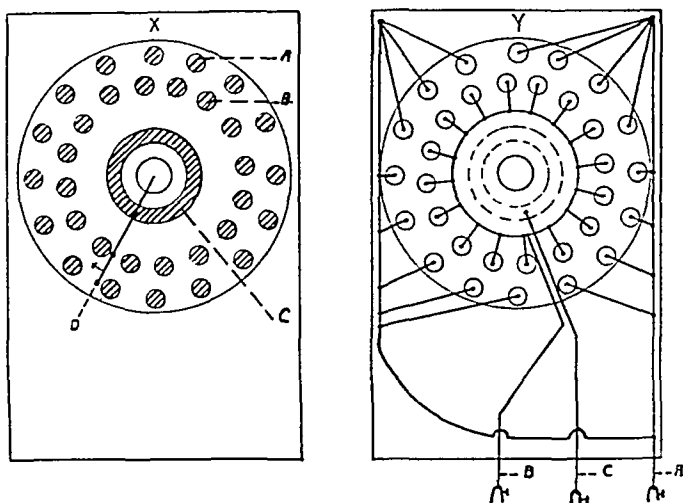


Fig 2—X Top of contact board A primary contact B intermittent short circuiting contact C constant short circuiting contact D drum carrying contact points Y Bottom of contact board A primary electrode B, intermittent short circuiting electrode C, constant short circuiting electrode

Fig 3 represents the manner by which the animal was maintained for activity and shows the electrodes in place.

The inset shows the electrode in detail.  $F$ , was the glass tubing housing the metal electrode. The glass housing was drawn in a tapering manner,  $C$ , over the entire metallic portion of the electrode. Such an arrangement permitted the adjustment of the tip,  $A$ , below the skin surface without shorting on the skin or subcutaneous tissue. The diameter of the housing was one-fourth of an inch and possessed a sufficiently large bore to permit the adjustment of the metallic electrode. After adjusting the metal the housing was heated and drawn to form a thin insulating covering over the metal.

The number 24 copper wire,  $E$ , was soldered at  $D$ , to a steel needle,  $B$ . The needle protruded from the housing at  $A$  for about one-eighth of an inch. The total length of the electrode was about 6 inches.

The electrodes were held in place by a twisted rubber band,  $H$ , which permitted unhampered movement of the electrodes. The rubber band,  $H$ , was in turn

supported by an insulated fork, *G*, which was fastened to an adjustable stand. When the electrodes were properly adjusted they appeared as indicated by *I* of Fig. 3.

Fig. 4 indicates the arrangement of the recording unit. The string, *J*, which was attached to the foot by a pin hook passed over pulley 1, and was fastened to the recording lever 1 cm. from the fulcrum. A continuation of the string descended from the recording lever and passed over pulley 2, and was attached to a calibrated spring, *A*. The calibration was equal to 1 gram per mm. The spring was initially set by the fixation of set screw, *D*, and the adjustment of the clamp *X*, so that the muscle was always after-loaded with a load amounting to exactly one-half of its own weight. The set screw, *D*, was then released.

It will be noted that the fixation of all parts of the recording apparatus em-

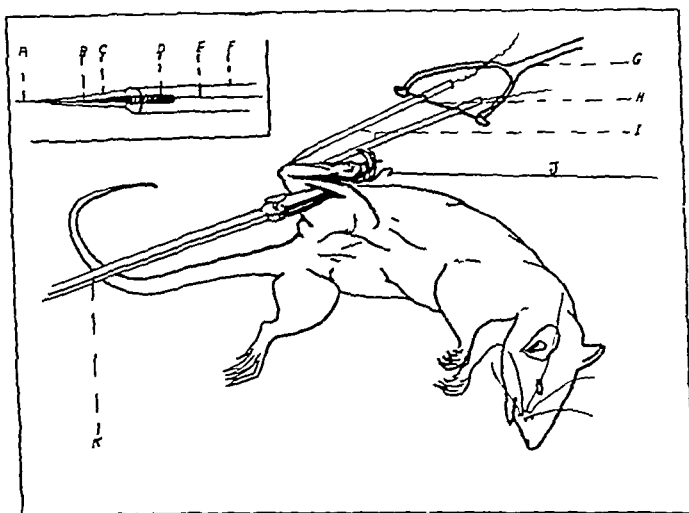


Fig. 3.—Adjustment of animal to apparatus. *A* Steel electrode point. *B* steel needle. *C* glass insulating shaft. *D* soldered joint between copper wire and steel electrode. *E* copper wire. *F* glass shaft. *G* electrode support. *H* rubber band. *I* electrodes in contact with animal. *J* string between animal's foot and recording unit. *K* clamp holding tibia.

phasized rigidity. The shaft, *C*, upon which the entire mounting took place was a three quarter inch bore steel pipe anchored to cement ceiling beams and to the cement floor.

The recording lever measured 15 cm. from the fulcrum to the tip of the paper writing point, with the string set 1 cm. from the fulcrum. The smoked recording paper was operated by two Harvard drums accommodated with a "ticker" for slow speeds. The length of the paper was measured so as to permit one complete revolution in about five hours.

The adjustment of the animal to the apparatus is shown in Fig. 3. When the electrodes were properly placed one was thrust into the *gastrocnemius* muscle and the other was inserted into the tendon *Achilles*. The leg was held in place by the clamp *K*, which was attached directly to the tibia. The only operative work upon the animal occurred here and amounted to a skin slit about one-half inch in length and the separation of the subcutaneous fascia as well as the bone fascia, so that the

tibia was readily accessible. Care was taken not to injure any blood vessel. Care was also used to insure the absence of any other structure in the clamp except bone. The clamp, *K*, was rigidly fastened to the work table and had absolutely no flexibility or vibration.

It is extremely essential to note that the tendon was not cut and that the set-up was in the nature of an eigographiæ technic so arranged that the after-loading of the muscle was taken care of by the natural anatomical arrangement of the bony levers. Such an arrangement was emphasized because of its physiologic naturalness.

The string, *J*, was fastened to the foot by a pin-hook which was always thrust

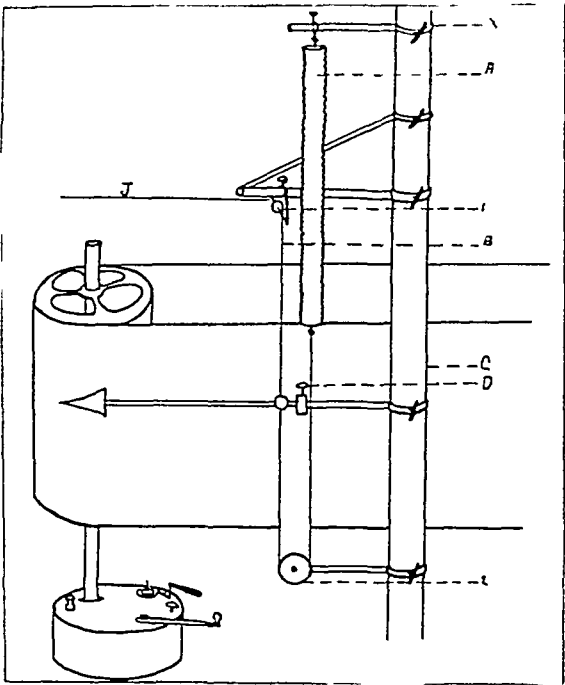


Fig 4—Recording unit. 1, spring; *B*, string attached to recording unit; *C*, 3/4-inch bore pipe; *D*, after loading screw; *J*, string as it passes from animal to apparatus; *X*, adjustable clamp; 1, pulley; 2, pulley.

TABLE I  
A SUMMARY OF THE KILOGRAM METERS OF WORK DONE PER GRAM OF MUSCLE BY THE AUTHOR'S AND THE CUT TENDON METHODS

AUTHOR'S METHOD		CUT TENDON METHOD	
	1.38		0.0262
	5.30		0.0290
	3.28		0.0290
	1.72		0.0631
	2.70		0.0285
	2.80		0.0647
	2.55		0.0527
	0.71		
Average	2.555		0.0419

through the middle phalangeal joints of the toes. The string was the best quality 20 pound test silk fish line. It was suspended from the ceiling with a 20 pound weight for ninety-six hours before use in order to take out all of the "stretch" which it might have had.

#### CONCLUSIONS

A method is described for studying the activity of the intact skeletal muscle of the anesthetized rat which permits much more work to be done by the muscle than when the usual methods are employed. This method permits one to retain the natural attachments of the muscle and thereby minimize disturbances caused by such conditions as over-stretching, diving temperature changes and interferences with the circulation and nerve control. A convenient automatic stimulating unit is described which permits a wide range in the choice of intensity and frequency of the stimulus. Most of the material required can be assembled from that usually found in a physiologic laboratory.

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## A HISTOCHEMICAL METHOD FOR THE DETECTION OF CHOLESTEROL\*

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PRACTICALLY all of the numerous sterol color reactions are based on the use of such anhydrous reagents as arsenic trichloride, acetic anhydride, or sulphuric acid. According to Rosenheim<sup>1</sup> the chromogenic properties of sterols in the presence of strong acids is due to the formation of carbonium salts which are colored. The colors produced are transitory and are discharged in the presence of moisture. Either the Salkowski or Liebermann-Burchard reaction in which concentrated sulphuric acid is used, is made the basis for most histochemical methods for the detection of cholesterol. They are all unsatisfactory, as the rapid play of colors is difficult to follow under a microscope, and the strong acid destroys the section within a very short time.

The physical characteristics of cholesterol preclude the possibility of the finding of a dye which will stain cholesterol within the cell. Fat soluble dyes are surprisingly few in number and unsatisfactory for the purpose. Unfortunately, stable chemical compounds of cholesterol which could be formed, are practically all colorless. Steidle and Kahlenberg<sup>2</sup> describe a dark brown substance formed by reaction with antimony pentachloride. This is an addition product of cholesterol and antimony pentachloride having the formula  $C_{27}H_{46}O \cdot SbCl_5$ . The reagent used in the following method consists of a 25 per cent antimony pentachloride solution in chloroform, to which a few crystals of antimony trichloride have been added in order to remove any free chlorine.

The procedure is as follows. The section is transferred to a slide, and blotted carefully three times with filter paper. It should not be absolutely dry. Treat with 1 or 2 drops antimony pentachloride reagent. A dark brown color develops instantly wherever the reagent comes in contact with cholesterol or its esters. Allow the excess reagent to evaporate. The white powdery substance which sometimes forms, disappears on blowing the breath over the slide. Wash with 1 or 2 drops chloroform, and mount in glycerol. Unsaturated fatty acids are stained a light orange, and may thus be differentiated from the dark brown of the cholesterol.

In very thin sections containing only traces of cholesterol, the fumes of antimony pentachloride may be used more satisfactorily than the chloroform reagent. A small glass rod is dipped in antimony pentachloride and then held over the section until the pentachloride fumes have thoroughly penetrated the tissue. This obviates the necessity of using chloroform which may remove the cholesterol. After the brown colored cholesterol compound has formed the section may be washed with a drop or two of chloroform, and the washings removed from the slide by means of filter paper.

\*From the Biochemical Laboratory of the Metropolitan Life Insurance Co  
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The brown compound of cholesterol is not readily soluble in chloroform. It does, however, dissolve slowly, yielding a deep purple color. If the section is mounted in chloroform, the deep brown slowly takes on a purplish hue, and thereby furnishes a further differentiation between cholesterol and unsaturated fats. When mounted in glycerol, a green brown color develops. There is no evidence of the color fading, even when the slide is exposed to sunlight for days.

The above procedure furnishes a rapid method for the detection of cholesterol in the various regions of the section. Unfortunately, it does not demonstrate cholesterol within the cell. The cell wall is either ruptured in the process, or there is a gradual diffusion of the coloring matter within the cell.

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## THE EXPRESSION OF HEMOGLOBIN CONCENTRATIONS ON A MOLAR BASIS\*

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THE adoption of a uniform method for the expression of hemoglobin values, in terms of grams of this pigment per hundred cubic centimeters of blood, may be considered an advance over the arbitrary "percentage of normal" values commonly employed. Through the medium of gram percentage, we may offset the differing standards employed by the hemoglobinometers in common use, and, provided these have been accurately standardized, we may correlate the results with different instruments and in different laboratories. The disadvantage of the "normal percentage" reading has been pointed out.<sup>1</sup>

Like all chemical substances present in blood, hemoglobin concentrations have a normal range rather than a normal fixed point, this range varying with various environmental factors. Whereas hemoglobin determinations in themselves are probably of more value than an isolated erythrocyte count, and repeated hemoglobin determinations, performed under identical conditions of blood dilution may be useful in following the course of hemorrhage or treatment, we have only one index as to the identity of blood dilution, a simultaneous red count.

The classification of anemias into macrocytic and hypochromatic varieties places more stress than ever on an accurate delineation of the ratio of pigment to corpuscular stroma. This is possible only where we have the hemoglobin concentration and the erythrocyte count at hand and much discrepancy between investigators might be avoided if their "normal" hemoglobin values were recalculated to an erythrocyte count of five millions. This is in effect a color index calculation. Any marked variation in the color index is significant of erythroblastic pathology,

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\*From the Laboratory of Clinical Pathology of the Chicago Free Dispensary and the Department of Physiology of the Chicago Medical School.  
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either the lack of adequate substances essential in mature corpuscular synthesis (high color index) or those necessary for pigment formation (low color index)

A general recognition of the utility of the color index is exemplified by the fact that the arbitrary "100 per cent" has persisted. Even in the newer forms of hemoglobinometers there is designated some particular concentration of the pigment to correspond with 100 per cent (excepting Haden's hemoglobinometer). There has been another, perhaps more potent reason for the retention of this latter figure. For years, in our courses in physiology and biochemistry we have tried to engender into our students, the propriety and necessity for accurate hemoglobin designations, only to see these same students, during their clinical years and internships, fall back into the fallacies of 100 per cent on no scale whatsoever.

For these reasons we have adopted the policy of allowing the 100 per cent to become 100 decimillimolar. By that maneuver "100" becomes a ponderable figure. Subsequently Avery and Hastings<sup>3</sup> (1931) pointed out the facility of expressing concentrations of other blood constituents on a molar basis. In the case of hemoglobin there is particular legitimacy for such expression. Where the physiologic rôle of a substance is as well understood as is that of hemoglobin, molecular designations give an immediate insight into the concentrations of those substances with which hemoglobin is identified. For instance, blood containing 100 decimillimolar hemoglobin is 100 decimillimolar in non-oxygen capacity and hematin as well (Table I). It remains to be seen whether the decimillimolar basis of expression is equal in exactitude to the gram percentage basis and whether it is a convenient figure for the calculation of color indices of human blood.

TABLE I  
EQUIVALENTS OF 100 DECIMILLIMOLAR HEMOGLOBIN IN CONCENTRATIONS OF

HEMOGLOBIN		HEMATIN		IRON		O <sub>2</sub> OR CO CAPACITY	
GRAMS %	ARBITRARY %	GRAMS %	MOLARITY IN DECIMILLIMOLS PER LITER	GRAMS %	MOLARITY IN DECIMILLIMOLS PER LITER	VOLUMES %	MOLARITY IN DECIMILLIMOLS PER LITER
16.67	Dare (old) 121.1 Fleischl 105.5 Talquist 105.5 Dare (new) 104.9 Barnard 100.0 Newcomer 98.6 Sghh 96.3	0.6396	100	0.05546	100	22.4	100

Hemoglobin differs from most proteins in providing excellent criteria for the determination of its molecular weight. Of these criteria we have:

1. The oxygen or carbon monoxide combining power. Since the ratio is always one mole of either of these gases to one mole of hemoglobin we may base a determination of molecular equivalency on the amount of oxygen or carbon monoxide chemically combined.

2. One molecule of hemoglobin can contain not less than one molecule of non-heme iron. From its non-heme content, Baercoft and Hill have calculated a minimum molecular weight of 16,669.

3 Osmotic pressure determinations by Adair<sup>2</sup> (1929), sedimentation velocity studies by Svedberg and Nichols<sup>13</sup> (1927) and determinations of diffusion constants by Northrup and Anson<sup>12</sup> (1929) have led to a calculation of the actual molecular weight of hemoglobin as being four times that reported by Hutner and Gausser<sup>11</sup> (1907) from their osmometer studies. There is apparently an association of four prosthetic radicals with globin in the formation of the molecule, but the equivalent weight, for practical purposes may be taken as 16,670.

4 Conant<sup>8</sup> (1923) and Conant and Fieser<sup>9</sup> (1924) have shown that hemoglobin may be titrated electrometrically with potassium ferricyanide. One iron equivalent of hemoglobin will reduce one mole of the oxidizing agent. The oxidation of hemoglobin to cyanmethemoglobin is easily followed potentiometrically and in this laboratory the method has been applied to the estimation of hemoglobin in blood (Fig 1).

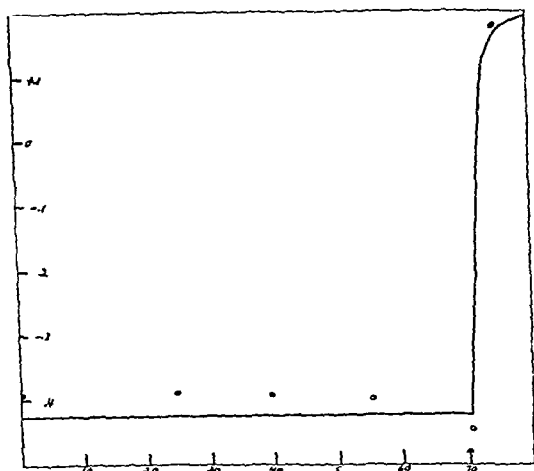


Fig 1—Electrometric titration of hemoglobin in blood. One cubic centimeter of blood containing 70 decimillimolar hemoglobin was centrifuged to throw down the corpuscles. The serum was discarded and the cells were hemolyzed with 10 c.c. 0.1 per cent NaCN solution. This was transferred to an electrometric titration vessel. 10 c.c. of ethyl ether was added and the sample titrated with 100 decimillimolar  $K_3Fe(CN)_6$  solution. Stirring was effected by a nitrogen inlet tube.

The abscissa represents decimillimols of ferricyanide  $\times 10^{-2}$  added; ordinates are potentials against a bright platinum electrode on the saturated KCl calomel scale.

5 The hematin content of hemoglobin, with our present exact knowledge of the constitution of hematin, may be used as a basis for estimation of the molecular equivalency of the latter.

In place of these five criteria for the molecular concentration of hemoglobin solutions, we have only one ultimate criterion for the gram percentage of such solutions, this depending on an accurate determination of the dry weight of the substance. In such a procedure there is always the question of (1) inadequate drying, (2) too extensive drying with decomposition of the molecule, (3) the identity of the globin moiety, and (4) admixture with other substances. Hemoglobin is a hydrated protein and one wonders whether it will yield this water, chemically combined or physically associated without dissolution. The globin fraction makes up 96 per cent of the molecule, and its constancy of composition is open to doubt. Finally, it is certain that those preparations of hemoglobin which were used to de-

termine some of the physical and chemical properties of the pigment, were not iso-electric, but actually sodium and potassium hemoglobins

On the basis of the work of Butterfield who gives the percentage of iron in human hemoglobin as being 0.0334 per cent and correcting for the amount of potassium which would have been associated with preparations crystallized at  $P_H$  7.4, we may take the concentration of a 100 decimillimolar hemoglobin solution as being 16.67 per cent. This is a convenient figure, since it is an aliquot of 100 and should it be taken as the 100 of a hemoglobinometer scale (Barnard,<sup>5</sup> 1932), the conversion of grams to decimillimols or vice versa is accomplished simply by multiplying or dividing by six.

TABLE II

HEMOGLOBIN ARBITRARY NORMALS FOR COLOR INDEX DETERMINATION RECALCULATED TO A DECIMILLIMOLAR BASIS

(100 DECIMILLIMOLAR = 16.67 GRAMS OF HEMOGLOBIN PER HUNDRED C.C. OF BLOOD)

HEMOGLOBINOMETER	STANDARD	METHOD	DECIMILLIMOLAR
Fleischl Miescher	Oxyhemoglobin		94.8
Tallquist	Oxyhemoglobin		94.8
Dare (new)	Oxyhemoglobin		96.0
Barnard	Cyanhematin		100.0
Newcomer	Hematin		101.6
Sihl	Hematin		103.8
			Average 98.5
<i>Authorities</i>			
Haden		Oxygen capacity	93.6
Barnard and Woolley <sup>1</sup>		Cyanhematin	97.9
Wintrobe <sup>2</sup>		Hematin	96.4
			Average 96.0

<sup>1</sup>Unpublished results

Calculated from data on mean corpuscular concentration and mean corpuscular volume (1931)

We must next inquire into the proximity of the 100 decimillimolar figure to the actual hemoglobin content of those samples of human blood which average five million red cells per cubic millimeter. Here there is recourse to data in the literature as well as to the "100" calibrations of those hemoglobinometers which are used in the calculation of color indices. It is quite obvious from Table II, that the 100 decimillimolar value, taken as the hemoglobin concentration to correspond to 100 per cent of erythrocytes, practically coincides with other values in use and for that reason its universal adoption is advocated.

#### SUMMARY AND CONCLUSIONS

It is suggested that the arbitrary 100 per cent scale of hemoglobin designation, as ordinarily employed for the calculation of color indices, be replaced by the ponderable decimillimolar scale. One hundred decimillimolar corresponds to 16.67 gm. per cent of hemoglobin and is approximately equal to the average concentration of the pigment in normal adult human blood averaging five million red cells per cubic millimeter.

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## AN INSTRUMENT FOR THE RAPID PREPARATION OF POTATO CYLINDERS FOR DIAGNOSTIC CULTURE PURPOSES\*

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THE use of potato cylinders for culture medium in bacteriologic practice dates back almost to the inception of bacteriology, yet this medium had not, until recently, proved of sufficient public health interest that the preparation of large numbers of cylinders from potatoes occupied an importance beyond that which could not be fulfilled by the ordinary laboratory equipment, such as a cork borer, and a small knife. Within the past few years, however, the potato cylinder has occupied a more important practical aspect in serving as the basis for a simple and rapid culture method for the diagnosis of tuberculosis in cases negative microscopically by the smear test†. Numerous requests for information regarding a suitable potato cylinder cutter prompted this brief description of an instrument devised and prepared by Mr Sam Pollak for use at the National Jewish Hospital at Denver about five years ago.

The instrument as shown in Fig 1 is prepared from a piece of brass tubing six inches long and five eighths of an inch in diameter. This size tubing cuts potato

\*From the Research Department, National Jewish Hospital at Denver  
Received for publication August 4 1932

†Corpor H. J. The Certified Diagnosis of Tuberculosis Practical Evaluation of a New Method for Cultivating Tubercle Bacilli for Diagnostic Purposes J A M A 91 771 1923  
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cylinders suitable for the commonly used six by five-eighths inch bacteriologic test tubes. If desired the cutter can be made of any size brass tubing suited to cutting potato cylinders to be used in any size bacteriologic test tubes. The lower cutting edge is sharpened similar to that of the usual cork borer and remains in tubular form for about one and one quarter inches after which the tubing is cut to one-half diameter for about three inches gradually tapering again at the top to the full diameter of the brass tube where the inserted hard wood handle cut on a slant and fastened with a small screw to the brass tube acts as an ejector for the cut potato

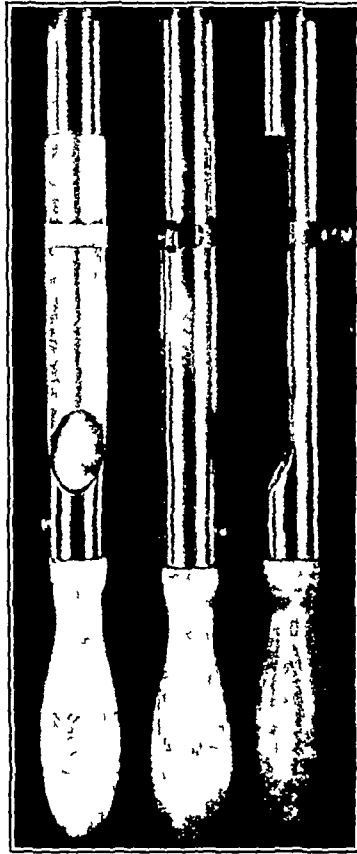


Fig 1

cylinders. About two inches above the cutting bottom edge a spring steel blade is bolted to the half diameter brass tubing for the purpose of halving the potato cylinders as they move up in the process of cutting. The nut and bolt clamp on the back of the spring steel cutter serves the purpose of adjusting this blade at any level desired by the technician. After the instrument has been suitably prepared the brass tubing is finally nickel plated to prevent corrosion and the hardwood handle is shellaced or varnished if desired. Properly cleaned and peeled potatoes after being suitably sized by cutting parallel pieces from opposite ends can be divided into halved cylinders about as quickly with this apparatus as the operator

can raise and lower his hand The potato for cutting is placed on a clean board covered with a suitable rubber pad which serves to protect the cutting edge of the potato cylinder cutter Any mechanic with a few tools and the necessary material can readily prepare a number of these cutters within a short time If properly handled and cleaned after each use they may serve for a period of several years in our experience

## A GRAPHIC METHOD OF DETERMINING CERTAIN NUMERICAL FACTORS IN METABOLISM\*

WALTER GOLDFARB, B S , NEW HAVEN, CONN

THE principle on which this method has been based is the same as that found in the papers of DuBois (1924) and Michaelis (1924) The determination of the numerical factors in metabolism in Michaelis' paper involves the use of two charts.

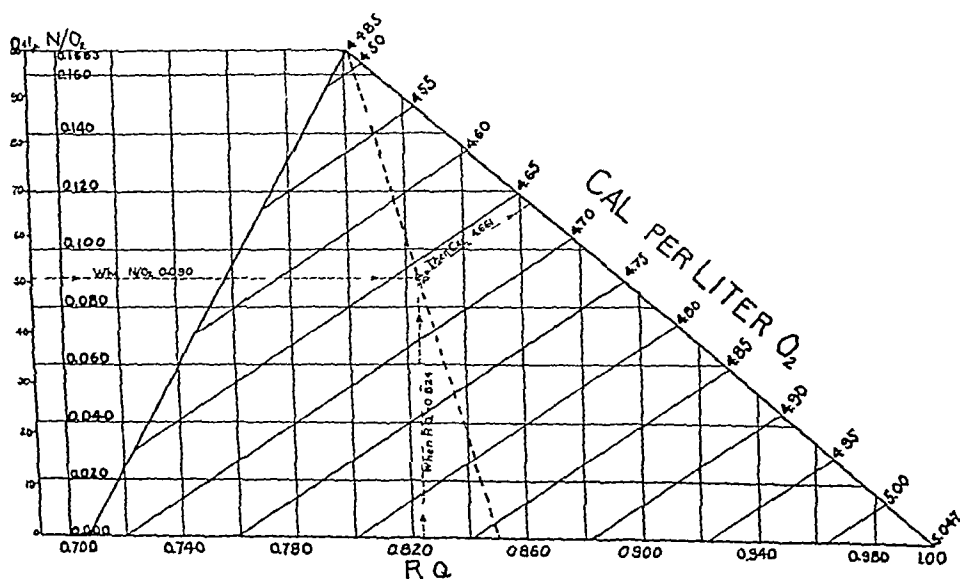


Fig 1—Chart showing the caloric value of a liter of oxygen according to the total R Q and the relationship of urinary nitrogen and total oxygen consumption (Modified from Michaelis Fig 2)

We have found it possible to obtain the same factors more readily using only the simpler of the two charts (Fig 1)

If the urinary nitrogen (in grams), the total oxygen consumption (in liters), and the total respiratory quotient are known we can obtain all the factors as follows The point P is obtained on the chart from the total respiratory quotient and the ratio  $\frac{\text{urinary-nitrogen (in grams)}}{\text{oxygen consumption (in liters)}}$  The caloric value of one liter of oxygen

\*From the Department of Physiology, School of Medicine, Yale University  
Received for publication August 1 1932

used for the mixture of foodstuffs is obtained by projecting P on the right side of the triangle parallel to the solid oblique lines drawn through the triangle. The percentage of the oxygen used for protein is read off the vertical scale on the left of the triangle. A line drawn through the upper vertex of the triangle and P will intersect the respiratory quotient scale at the base at a point equal to the nonprotein respiratory quotient. Now

- $$(1) \text{ \% calories from protein} = \frac{\text{\% O}_2 \text{ for protein} \times 4.485}{\text{calories per liter O}_2}$$
- $$(2) \text{ \% calories from carbohydrate} = \frac{(100 - \text{\% cal from protein}) (\text{\% cal from carbohydrate at nonprot R Q})}{100}$$
- $$(3) \text{ \% calories from Fat} = 100 - (1 + 2)$$

The only change made in Fig. 2 of Michaelis' paper is the line drawn through P and the upper vertex of the triangle. The constants used are the same.

For purposes of demonstration the same example used in Michaelis' paper is chosen. The ratio  $\frac{N}{O_2}$  has been taken as 0.090, and the R Q at 0.824.

$$\begin{aligned} \text{\% O}_2 \text{ for Protein} &= 53.5 \\ \text{Calories per liter O}_2 &= 4.661 \\ &53.5 \times 4.485 \\ \text{\% cal protein} &= \frac{240.5}{4.661} = 51.5 \\ \text{nonprotein R Q} &= 0.850 \\ &48.5 \\ \text{\% cal carbohydrate} &= \frac{48.5}{100} (50.7^*) = 24.6 \\ \text{\% cal fat} &= 100 - (24.6 + 51.5) = 23.9 \end{aligned}$$

#### REFERENCES

- Du Bois, E. F. Clinical Calorimetry, Graphic Representation of Respiratory Quotient and Percentage of Calories From Protein, Fat and Carbohydrate, *J Biol Chem* 59: 43, 1924.  
 Michaelis, A. M. Clinical Calorimetry, Graphic Method of Determining Certain Numerical Factors in Metabolism, *Idem* 59: 51, 1924.

\*Constants at R Q 0.850 from Lusk. *Science of Nutrition* fourth edition 1928 p. 65



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## VINCENT'S ORGANISMS, Pathogenicity of the Fusiform Bacillus and Spirillum of Plaut-Vincent, Lichtenberg, H. H., Werner, M., and Lueck, E. V. J. A. M. A. 100 708, 1933

Attempts at producing lesions in any way similar to those commonly attributed to the action of the Plaut Vincent organisms by injecting pure cultures of fusiform bacilli into areas of traumatized tissue in guinea pigs were unsuccessful.

The fusospirochetal organisms were found in 45.4 per cent of tonsils removed from 108 children.

In the same children these organisms were found in 91 per cent of the membranes that formed over the tonsillar beds after tonsillectomy, and usually in greater numbers than in the tonsils themselves.

The organisms were found constantly in smears of the membranes that formed over traumatic ulcers produced in the mouths of guinea pigs.

Neither the injection nor the local application of sulpharsphenamine hindered the appearance of these organisms in the lesions in the mouth of guinea pigs or hastened the healing of the lesions.

Sixteen consecutive cases of severe ulcerative stomatitis in children all healed in some four to seven days without treatment. This compares favorably with the reports of cases treated with various drugs and other forms of treatment.

The value of diagnostic smears for Vincent's organisms as a means of establishing a pathogenic relationship of these organisms to a suspected lesion is questioned.

## SPINAL FLUIDS, Cell Count and Encapsulation of Brain Abscess, Woltman, H. W. J. A. M. A. 100 720, 1933

Abscess of the brain presents a highly varied clinical panorama that cannot be reduced to a simple formula. Each case is a law unto itself. The problem calls not only for a diagnosis but also for decision as to when the abscess shall be drained. Awaiting the optimal time means a better capsule and a liquefied interior, and consequently better drainage, it means less virulent organisms and greater immunity, and hence less danger to the patient.

The risk of performing spinal puncture in cases of abscess has probably been overrated, and thus physicians have been deprived of information that might be helpful.

After invasion of the brain has taken place and the formation of an abscess gets under way, the number of neutrophils in the spinal fluid becomes absolutely and relatively reduced.

The persistence or reappearance of neutrophils suggests that encapsulation is not progressing favorably.

An appreciable number of neutrophils may indicate extension of the abscess or close proximity of the abscess to the ventricle.

A predominance of neutrophils in the spinal fluid was seen in cases in which operation was performed unnecessarily or which ended fatally.

A small number of lymphocytes would seem, on the whole to indicate better encapsulation, greater resistance, and a smoother convalescence after operation.

## VACCINE THERAPY, A Study of Pathogen Selective Cultures in Relation to, Boerner, F., and Solis-Cohen, M. Am. J. Clin. Path. 3 125, 1933

A series of 401 pathogen selective cultures from 150 patients were studied.

In many cases the isolation of the more important pathogenic organisms was aided by the bactericidal action of the patient's fresh, whole, coagulable blood upon the less important and presumably nonpathogenic organisms.

Information was obtained regarding the presence or absence of bactericidal substances in the patient's blood for the various organisms isolated

In about 11 per cent of the cases organisms were isolated which would have been missed by the ordinary methods of culturing

A basis is provided for selecting the organisms to predominate in an autogenous vaccine, on the assumption that the organisms which grow in the patient's fresh, whole, coagulable blood are of most importance to the individual

**P TULARENSE, A New and Simplified Medium for, Rhamy, B W** Am J Clin Path 3 121, 1933

Formula of dehydrated "Bacto Cystine Heart Agar" (Difco)

Beef Heart, infusion from	500 gm
Bacto peptone	10 gm
Bacto dextrose	10 gm
Sodium chloride	5 gm
L cystine	1 gm
Bacto agar	15 gm

Reaction  $P_{H}$  6.8 (The reaction may range between 6.8 and 7.3 but 6.8 gave the most luxuriant growth)

Both the Bacto cystine heart agar and Bacto hemoglobin can be obtained in dehydrated form as a stock supply

Method for preparing 500 c.c.

(A) "Double Strength Agar"—Dissolve, by boiling 28 grams Bacto cystine heart agar in 250 c.c. distilled water. Sterilize for twenty minutes at 15 pounds' pressure (250° F). The sterile agar will be dark in color (chocolate agar)

(B) Dissolve 5 grams Bacto hemoglobin in 250 c.c. distilled water and strain through gauze to remove any large undissolved particles. Sterilize twenty minutes at 15 pounds' pressure

(C) Cool both of the above sterile solutions (A and B) to 50° to 60° C and mix. Dispense in sterile test tubes or other containers. Use strictly aseptic conditions. Incubate to test sterility

**AGGLUTININS, A Study of O and H Agglutinins in Typhoid and Endemic Typhus Fever, Kemp, H A.** Am J Clin Path 3 135, 1933

Alcoholized E typhi were used as O agglutininogen in agglutination tests carried out serially in six cases of typhoid fever. Positive agglutination was observed earlier than is usually observed in the Widal test

Growth phase selected O agglutininogen was positively agglutinated later than is usually found in the Widal reaction

Formolized growth phase O agglutininogen was agglutinated even later than untreated growth phase selected agglutininogen and at much lower titers

Both O and H agglutinins were found in the sera of typhoid paratyphoid vaccinated individuals. The same individuals did not have O agglutinins prior to vaccination

Both O and H agglutinins were found in the sera of individuals suffering with acute exacerbations in chronic arthritis

Both O and H agglutinins for B proteus X 19 were found in the serum of seven cases of endemic typhus (north Texas)

**SEPTICEMIA, In the Newborn, Dunham, E C** Am J Dis Child 45 229, 1933

Septicemia, in spite of the general impression to the contrary, is an important and relatively frequent cause of morbidity and mortality in the newborn. Blood cultures should

always be taken when a newborn infant becomes ill and the diagnosis is obscure. If the cause of the illness is determined early and transfusions of blood as well as other treatment are given, a number of these infants may recover.

Thirty nine cases of septicemia in newborn infants occurring in a period of five years in the New Haven Hospital are reported. The final diagnosis, based on the growth of organisms from the blood, was usually corroborated by clinical, and, in many cases, by post mortem examination. The commonest organisms found were the streptococcus, the staphylococcus and *B. coli*.

Clinically septicemia due to the streptococcus seems to differ from other types of septicemia by the absence of jaundice and bleeding, by the less frequent enlargement of the spleen and by the more frequent appearance of cutaneous infections, omphalitis, peritonitis and meningitis. Streptococcus septicemia invariably had a fatal outcome.

In the cases of septicemia caused by the staphylococcus or by *B. coli*, on the other hand, jaundice was a common symptom, occurring in more than one half of the cases in both groups. Bleeding took place in about one half of the cases in the staphylococcus group and in about one third of the cases in the *B. coli* group. The spleen was frequently enlarged (one half or more of the cases) in both groups. Anemia, however, was less common in staphylococcus septicemia than in that caused by the streptococcus and *B. coli*. Infection of the urinary tract was found only in cases of *B. coli* septicemia. Staphylococcus or *B. coli* septicemia was not invariably fatal.

#### **MALARIA, A New Method for Staining Thick Films, Chorine V. Bull. Soc. Path. Exot. 25 651, 1932**

Dry at a low temperature. Fix in 10 per cent commercial formalin until dehemoglobinization is complete, three to fifteen minutes. Wash. Treat with a solution consisting of 3 c.c. of Lugol in 100 c.c. of a 2 per cent solution of potassium iodide, for three to five minutes. Wash. Stain in Giemsa, 2 drops to each 1 c.c. of hard tap water, for one hour. The formalin dehemoglobinizes and fixes at the same time. It is advisable to add calcium carbonate to the formalin, to neutralize acidity. The optimum  $P_{H}$  is 7.3 to 7.5.

#### **BILIRUBIN, SERUM. Relationship Between the Bilirubin Content of Serum From Different Types of Jaundice and the Icterus Index of the Serum After Removal of Proteins, White, F. D. Brit. J. Exper. Path. 14 17, 1933**

The residual icterus index is the name given to the icterus index when determined on serum from which the proteins have been removed by precipitation with alcohol.

It has been shown that in serum artificially rendered icteric by the addition of bilirubin, the residual icterus index is directly proportional to the bilirubin content in mg. per 100 c.c., the ratio being approximately 6 to 1.

Results have been obtained indicating that within the limits of this investigation, this relationship also holds for the serum of normal individuals and of jaundice cases of hemolytic origin, but does not hold for other types of jaundice.

The icteric ratio of serum is defined as the ratio of the icterus index to the residual icterus index.

In normal serum and icteric serum of hemolytic origin this icteric ratio has been found to be generally less than 1.2, whereas in all other jaundice cases investigated it was almost invariably much higher. Since this ratio is easily and quickly determined, it is suggested that in cases of latent or slight jaundice it might prove of assistance in differentiating such cases into "hemolytic" and "nonhemolytic" categories. A further differentiation of the latter category into "obstructive" and "toxic" types has not been found possible by this method.

It is claimed that the generally accepted normal values for serum bilirubin are too low and that the normal range is at least 0.5 to 1.0 mg. and possibly 0.5 to 1.5 mg. per 100 c.c. serum.

**PNEUMOCOCCUS, Studies of the Skin Reactions to the Specific Soluble Substances of the Pneumococcus Types I and II.** Alston, J. M., and Lowdon, A. S. R. Brit J Exper Path 14 1, 1933

In 281 persons who had not recently suffered from a pneumococcal infection, sensitiveness (increasing with age) to intracutaneous injection of pneumococcal SSS type II was found in 63 per cent

Reactors retested after five to twelve months were still sensitive in 92 per cent of cases

Repeated tests at short intervals always produced a decrease, and in some cases a complete loss of sensitiveness

In animals and human beings actively or passively immunized to pneumococci no immediate reactions could be produced by SSS of homologous type. In rabbits, however, vaccination caused the development of a delayed form of reaction to the carbohydrate

Skin sensitiveness (for immediate reactions) to the pneumococcal type specific carbohydrates and circulating serum antibodies can each occur independently of the other and this work does not support the suggestion that the skin reactions are associated solely with the recovery from pneumonia

In the normal persons tested with intracutaneous injections of SSS type II a considerable proportion showed as well as, or independent of, the immediate reaction, a delayed reaction. This was also found to be much more frequent in the older persons

**PELLAGRA, Erythrocytes In,** Turner, R. H. Am J M Sc 185 381, 1933

Using the most accurate available methods, erythrocyte counts and determinations of hemoglobin and percentages of packed red cells have been made for 70 bloods from 50 patients with typical pellagra

Of the pellagrins 56 per cent showed no appreciable anemia, 16 per cent slight or questionable anemia, 12 per cent showed moderate anemia and 12 per cent a severe anemia, while 4 per cent showed an extremely severe anemia

Two thirds of the patients who died showed no anemia according to the methods and standards used

Among those with severe anemia, other diseases which might have caused anemia were common

Patients who suffered from diarrhea did not appear to be more anemic than those without diarrhea

The influence of dehydration in obscuring anemia is discussed

Anemia, when present, was definitely of the chlorotic, normocytic, or microcytic type, and in no instance of the macrocytic type

Of the pellagrins 34 per cent had erythrocytes with corpuscular hemoglobin concentration less than normal, while for 66 per cent the concentration was within the normal range

The average size of the red cells tended to diminish in proportion to the severity of the anemia, contrasting with the opposite rule for pernicious anemia

The importance of the blood picture in the differential diagnosis between pellagra and pernicious anemia or sprue is emphasized

**LEUCOCYTES, Nonfilament Polymorphonuclear Neutrophil Count in Typhoid and Undulant Fever,** Gallagher, J. R. Am J M Sc 185 391, 1933

Total white blood cell counts and nonfilament polymorphonuclear neutrophil counts are recorded in 17 cases of typhoid fever and in 2 cases of undulant fever. These show a striking increase in the number of nonfilament, or immature, forms in the majority of instances

The value of the nonfilament form count in the estimation of bone marrow response in these diseases is emphasized

The suggestion is made that the increase in the nonfilament form of polymorphonuclear neutrophilic leucocyte is evidence that the leucopenia in these diseases is associated with white blood cell destruction rather than with bone marrow inhibition, but attention is

directed to the possibility that the increase in the number of young forms present may be a result of a bone marrow disturbance which inhibits the maturation of the neutrophils

**LEUKOCYTES, Filament-Nonfilament Count in Chronic Arthritis, Steinbracher, O., and Hartung, E F J Am M Soc 100 654, 1933**

The filament nonfilament count is a useful routine diagnostic aid in chronic arthritis

Filament nonfilament counts in fifty patients with rheumatoid or chronic infectious arthritis were abnormally elevated in 100 per cent of the patients

The filament nonfilament count was normal in twenty six patients, or 52 per cent, of a group of osteo arthritic patients, while in the rest of this group the count was elevated

The average nonfilament count was much higher (31.5 per cent) in patients with rheumatoid arthritis than in osteo arthritic patients with an abnormal count (22.3 per cent)

The filament nonfilament count is helpful in differentiating rheumatoid arthritis from osteo arthritis only when within normal limits. A normal count indicates that chronic rheumatoid infection is not present. An elevated count may indicate the presence of rheumatoid arthritis, mixed rheumatoid and osteo arthritis, or osteo arthritis with active focal infection

**TUBERCLE BACILLI Comparison of Certain Media for Cultivation of From Sputum, Shaffer, M. F. Am Rev Tuberc 27 259, 1933**

In this study 6 media have been compared as to their relative value for the cultivation of the tubercle bacillus from sputum, following preliminary treatment of the 42 specimens with 6 per cent sulphuric acid according to the slightly modified Corper Uyei method

The medium of Corper and Uyei yielded the largest number of positives, being equal to the microscopic method in total number of results. Because of simplicity of preparation and the excellent results obtained through its use, this medium is particularly recommended for primary cultures of the tubercle bacillus following acid pre treatment

The medium of Petragham is likewise highly recommended, since it yielded nearly as good results as those obtained with the Corper Uyei medium. These two were superior to the other four media tried in numbers of positives, at all times

Lubenau's medium proved slightly superior to the media of Dorset, Petroff and Sweany Evanoff, all of which gave practically the same results

In this series the culture method has proved itself to be at least the equal of microscopic examination and, if anything, slightly superior to it. Out of 42 specimens examined, the microscopic method showed 33 positives while the culture tubes revealed 37 positives

In the routine isolation of tubercle bacilli from sputa, it is recommended that both the medium of Corper Uyei and that of Petragham be employed

Dissociation of the primary cultures into colonies resembling the so called R and S types has been noted particularly on Petroff's medium

**TISSUE Rapid Method for, Erskine, E B J A M A 100 573, 1933**

Fresh tissue is cut into convenient size and about 2 mm thick. This tissue is placed in a test tube of neutral "solution of formaldehyde U. S. P.," diluted 1:10 and gently boiled for a period of three minutes, during which time five test tubes, appropriately labeled and containing respectively (1) 80 per cent alcohol, (2) 95 per cent alcohol, (3) 100 per cent alcohol, (4) chloroform and (5) 95 per cent alcohol are placed in a water bath and the temperature brought to 55° C.

Temperature is accurately gaged by a thermometer placed in the test tube prior to placing the tissue in the tube, whereupon the thermometer is dried and placed in the succeeding tube solution. It will be noted later that Tube 5, containing 95 per cent alcohol, serves no purpose other than a check on the temperature during the time the tissue is in Tube 4.

The fixation of the tissue in boiling formaldehyde solution having been completed, the

solution is poured off and the tissue dried on blotting paper. It is then placed successively in the solution contained in Tubes 1, 2, 3 and 4, for a period of five minutes in each.

The next two steps are carried out in the embedding oven at the same temperature (55° C). Chloroform and paraffin (equal parts) and paraffin are always kept in the oven at the temperature mentioned and consequently are always available for this method. The tissue is kept in each solution for five minutes, whereupon it is embedded in paraffin, immediately chilled in a pan of ice water, cut and mounted on a slide.

#### **ANEMIA, Primary Hypochromic, Dameshek, W. J. A. M. A. 100 540, 1933**

The clinical features of twenty five cases of primary hypochromic anemia were studied.

The disease is characterized by the presence in adult women of a chronic idiopathic "secondary" anemia, subject to remissions and relapses.

It presents the following clinical features:

*Symptoms*—Weakness, dyspnea, gastrointestinal complaints, sore tongue, paresthesias, weight loss.

*Signs*—Pallor without icterus, wrinkled, atrophied, inelastic skin, glossitis, brittle, often spooned, finger nails, dry, gray hair.

*Blood*—Anemia or low color index, hypochromia of the red blood cells, small average red blood cell diameter, leucopenia, and reduction in blood platelets.

*Gastric Juice*—Achlorhydria, usually complete, small volumes, increased mucus.

*Response to Medication*—Prompt and striking response to large doses of inorganic iron, small doses of copper sulphate of benefit when the anemia becomes "fixed."

Certain cases are associated with pregnancy, dysphagia (Plummer-Vinson syndrome), gastrectomy and gastroenterostomy, myxedema, and tapeworm infestation. The common feature is achlorhydria.

On the basis of striking response to treatment with inorganic iron, there is a possibility that an iron deficiency is present. It is possible that absence of free hydrochloric acid from the gastric juice may result in poor absorption of food iron from the gastrointestinal tract.

#### **Anemia of Pregnancy, Rowland, V. C. J. A. M. A. 100 537, 1933**

Liver extract is specific in pernicious or hypochromic anemia of pregnancy. The anemia is due to a relative or temporary deficiency of a specific hemogenic substance produced in normal gastric digestion. This substance is apparently identical with that lacking in true Addisonian anemia.

Iron in dosages of from 90 to 120 grains a day is specific in the secondary or hypochromic anemia of pregnancy. This anemia is probably due to dietetic deficiency and to faulty absorption of the hemogenic substance on account of gastrointestinal disturbances.

These anemias occur in slight degree in the majority of American women during pregnancy and are associated with hypochlorhydria and a deficiency in the specific intrinsic factor involved in blood formation.

The mechanism of hematogenesis, ordinarily quite adequate, is apparently overstrained by the metabolic overload of pregnancy and likewise by various infections, toxic agents, and marked nutritional deficiencies.

Pernicious anemia can hardly be regarded as one specific disease but rather as a non-specific failure of hematogenesis, due to a variety of causes that operate through the common mechanism of a deficiency or disorder of gastric function.

In the rare case of severe pernicious anemia of pregnancy, the intramuscular or intravenous use of liver extract may be life saving either before or after delivery. The response may be noted within thirty six hours. If the patient survives the first five days, she may be expected to recover.

Transfusion is useful as an emergency measure only.

Termination of pregnancy is indicated mainly by serious complications.

Obstetric morbidity and mortality statistics might be improved by applying the newer methods of treatment and prophylaxis in anemia in prenatal work in order to bring the blood up to full normal.

**TRANSFUSION, Prevention of Chills Following Transfusion of Citrated Blood, Lewisohn, R., and Rosenthal, N J A M A 100 466, 1933**

The authors emphasize the necessity for a centralized department for the thorough cleansing and preparation of transfusion apparatus for the citrate method, report the practical elimination of chills when this is done, and describe their method as follows

1 *Distilled Water, Sodium Citrate and Sodium Chloride*—Triple distilled water, obtained from a special Barnstead still, is used for the preparation of sodium citrate (30 per cent) and sodium chloride (0.85 per cent)

Sodium citrate (30 per cent) may also be bought on the open market in 5 c c ampules. One should inquire from the manufacturer for sodium citrate prepared with triple distilled water. For actual use, 1 c c of this concentrated sodium citrate solution is used for every hundred cubic centimeters of blood.

2 *Special Cleansing of Apparatus*—After each transfusion, all parts are separated and washed in cold water for the removal of blood. They are then washed in a dilute solution of green soap to which compound solution of cresol has been added to make up about a 1 per cent solution. They are then thoroughly rinsed in tap water.

All parts are then placed in a large pan containing sodium hydroxide (0.1 per cent solution) and boiled for five minutes. They are then transferred to a large pan containing distilled water, to remove the sodium hydroxide. The glassware and rubber tubing are again washed with triple distilled water and are ready to be assembled and sterilized, either in metal boxes or in special bundles in the autoclave.

The glassware and rubber tubing are boiled separately. The needles are always sharpened before being treated but boiled for only three minutes in sodium hydroxide solution (0.1 per cent).

3 *Preparation of Apparatus for the Autoclave*—A For the donor: A bundle containing the apparatus for taking the blood from the donor (two pyrex cylinders 500 and 10 c c, respectively, one glass rod, two tourniquets, two needles gage 13 and 15, Luer adapter and rubber tubing, one ampule of 30 per cent sodium citrate).

B For the recipient: A metal box, containing glassware, rubber tubing and cannulas for the transfusion into the recipient.

**Aschheim Zondek Test, Modification, Budd, G V, and Ingram, W W Med J Australia 2 629, 1932**

The following modification enables the test to be read in 55 to 72 hours.

The early morning specimen of urine, slightly acid to litmus, is mixed with three volumes of absolute alcohol and allowed to stand for thirty minutes. The mixture is centrifuged and the supernatant fluid is poured off. The precipitate is taken up in a small volume of water and the mixture is again centrifuged. The aqueous extract is found to be highly active. The precipitate may be spun down in a 100 c c centrifuge tube in three spins, the supernatant fluid being discarded each time. The supernatant fluid is decanted off and the tube drained for a few minutes, or, if the precipitate is bulky, the tube is placed in a water bath at 40° C and exhausted for a few minutes in order to remove most of the alcohol. The precipitate is taken up with 1.5 c c of distilled water and transferred to a 10 c c centrifuge tube. The large centrifuge tube is washed out with three quantities of 0.7 c c of distilled water and the washings added to the 10 c c centrifuged tube. After thorough mixing, the insoluble portion is centrifuged out. The concentration effected by the procedure is about 15 to 1. After preliminary tests with the concentrates prepared in this manner, the preparation of the concentrate from urine was commenced in the morning. Four mice, from twenty-eight to thirty days old, receive two injections of from 0.3 to 0.4 c c at an interval of six hours. Fifty-five hours after the first injection, the ovaries of two of the mice were examined. If hemorrhagic follicles were not seen, the remaining mice were examined about seventy hours after the first injection. The finding of one hemorrhagic follicle constitutes a positive reaction. In the absence of hemorrhagic follicles the result of the test is "negative."

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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### Specific Changes in the Blood Serum

**B**ENDIEN will be recognized as the originator of a recently described serologic test for malignancy based upon a disturbance of serum lability in the presence of a malignant process and elicited by flocculation of the serum in the presence of varying concentrations of orthosodium vanadate and tenth normal acetic acid

In this volume is presented a short account of the researches upon which the test is based. The book is not intended as a manual for the laboratory worker and presents the subject in broad outline rather than in detail.

While the results reported by Bendien have not as yet been corroborated by other workers, the work presented represents extensive and serious scientific study and as such deserves the consideration of all concerned with the subject.

The volume should be of interest for its contribution to the study of malignancy and as such demands the attention of workers in this field.

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### The Microbiology of Foods†

**T**HIS is a book which undoubtedly will take a foremost place on the working shelf of the laboratory worker and will soon acquire the honorable scars of frequent use.

Microbiology as concerned with the desirable and undesirable activities of bacteria in their relation to foodstuffs is a branch of bacteriology which has undergone extensive expansion within recent times.

Much of the information, practical as well as theoretical, which has accumulated as a result of the numerous and varied studies in this field have not been readily accessible either to the laboratory worker or the physician in general. Now, in this book, it has been gathered, reviewed, summarized, and presented in an orderly, systematic, and comprehensive manner.

In its presentation of the subject this volume is eminently practical.

Whether one wants to know what studies have been made of foodstuffs or the manner in which they may be acceptably prepared commercially, or what constitutes a satisfactory standard, or how to determine if a particular sample conforms to this standard, all will be found clearly outlined and discussed.

The subjects of food poisoning and food infection, while of course referred to are not discussed in detail, this being reserved for a separate volume.

As said above, the book may well take its place as a standard reference to be read with profit by the physician as large as well as the laboratory worker.

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### Primary Syphilis in the Female

**T**HIS volume is based upon the experience of the author as Supervisor of the Venereal Clinic at St. Thomas' Hospital, London, since 1917.

Primary syphilis in the female is not only rarely seen by the average practitioner but has not been subject to any extended or detailed account in the literature. This volume, therefore,

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\*Specific Changes in the Blood Serum. By S. G. T. Bendien translated by A. Piney. M.D. Cloth 95 pages 64 illustrations. The C. V. Mosby Co. St. Louis Mo.

†The Microbiology of Foods. By F. W. Tanner Professor of Bacteriology, University of Illinois. Cloth 768 pages. The Twin City Printing Co. Champaign Ill.

\*\*Primary Syphilis in the Female. By Thomas Anwyl Davies. Cloth 111 pages 24 figures and 12 colored plates. Oxford University Press.



fills a needed gap and does so in an excellent and comprehensive manner. Attention is called to the fact that of 584 primary genital chancres in the female, 45 per cent were internally situated, 44 per cent being on the cervix which, therefore, should be examined as a routine.

The typography, arrangement of the material, and the illustrations are excellent, the whole making a very valuable book worthy of a place in the library of every physician.

### Laboratory Service and the General Practitioner

**T**HIS book is not intended to be, nor is it, a manual of laboratory technique, but has for its purpose the interpretation and application of laboratory work in clinical purpose.

Books of this kind are sorely needed and the present volume, though small, should be welcomed by the physician for the information it gives of how and when to utilize the methods of the laboratory and how to interpret in terms of clinical applicability to the patient, the reports rendered.

An appendix contains an alphabetical list of disease and the most informative laboratory examinations applicable.

The book deserves a warm reception by the practitioner.

### Behavior Aspects of Child Conduct

**B**EHAVIOR is a word implying genetic interest and conduct a word implying a moral and philosophic interest in a person's actions.

The views of former generations that annoying behavior in a child was due solely to "badness" or nervousness have largely been replaced by those regarding behavior as an aspect of mental or physical health.

This concept has been so confused by a multitude of psychological and psychiatric theories and so buried in a bewildering mass of words that this practical and understandable volume by Dr. Richards will be received with sincere gratitude by its readers.

The book embodies a series of lectures delivered under the auspices of the Child Association of America and designed, not so much to present or enter into theoretical discussions, as to present some help in the solution of individual behavior problems.

This purpose is well and satisfactorily achieved and the book can be recommended for lay as well as professional readers.

### Public Health in New York State

**I**N 1930 Governor Franklin D. Roosevelt of New York appointed a Special Health Commission to survey and report upon the administrative and legislative aspects of public health and to make whatever recommendations it might deem advisable to lessen, by means of better utilization of existing knowledge, the gap between what is achieved and what might be realized in the conservation of public health.

In this volume is presented the detailed and comprehensive report of the commission.

It may well be read by all concerned with the problems of public health for its wide vision, careful and thorough presentation, and the value of the recommendations made.

\*Laboratory Service and the General Practitioner. By Arnold Penshaw. Director, Laboratory of Applied Pathology and Preventive Medicine, Manchester etc. Cloth 267 pages 7 shillings. Oxford University Press.

†Behavior Aspects of Child Conduct. By Esther L. Richards. Associate Professor of Psychiatry, Johns Hopkins School of Medicine. Cloth 299 pages. The Macmillan Co. New York.

\*\*Public Health in New York State. Cloth 704 pages 37 charts numerous photographs. New York State Department of Health. Albany.

The example set by the appointment and work of this commission could be very profitably followed by the country at large

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The conclusion appears justified that, in spite of a few exceptional cases, the efficacy of immunization may be deduced from the change of the Schick reaction from positive to negative

Taking as a basis the figures concerning the morbidity, the percentage of Schick reactions rendered negative after the injections of the immunizing agent, and a certain number of titrations

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or antitoxin in the blood of persons immunized, it may be concluded that formol toxoid (anatoxin) is the most efficient of those antigens which have been the subject of our comparative study, i. e., mixtures of toxin, antitoxin and formol toxoid (anatoxin), and should be recommended for the present

The clinical observations submitted to the conference indicate that, so far as can be determined up to the present, the immunizing power of formol toxoid (anatoxin) in man appears to be in relation with the antigenic value of the prophylactic as measured by the flocculation method

The method of administration recommended is by subcutaneous injection. When this is not possible, the prophylactic may be applied to the nasal mucous membrane. Our experience with the cutaneous method has not been favorable

Immunization should be carried out by means of three injections. It is hoped that, in the future, the use of a more active prophylactic will make it possible to immunize with two or perhaps even one injection

The intervals recommended between injections are three weeks between the first and second injections, and at least two weeks between the second and third

It is unnecessary to carry out the Schick reaction before immunization. It is, however, desirable to use the Schick reaction before and after immunization in a certain proportion of subjects in order to determine the value of the method employed

It is recommended that immunization against diphtheria should be carried out not later than during the pre school period, after the end of the first year of life

If the children have not been immunized during the pre school period, they should be immunized, if possible, during the first year of school attendance

Charitable organizations and administrations receiving children in homes (holiday camps, "preventoria," sanatoria, etc.) are advised to require, from both children and staff, a diphtheria immunization certificate on admission or a certificate attesting that the Schick reaction was negative

Immunization is recommended for medical, nursing and domestic staff in hospitals, homes, dispensaries, sanatoria, schools, etc.

Immunization is to be recommended even during epidemics in the case of children who have been in contact with patients, as there is so far no evidence on record as to the existence of a negative phase

In the opinion of the experts, diphtheria immunization should form the subject of active public education on the part of the health administrations of the different countries in order to bring home to everyone the advantages of this method of protection in safeguarding the public health

The report and study of Medical Education in England by Sir George Newman, Chief Medical Officer of the Ministry of Health and of the Board of Medical Education, England, is of extraordinary interest and value. It is regrettable, indeed, that this study cannot be made generally available for it should be read, not only by all associated with medical education, but by all physicians

The views expressed are based upon an extensive practical experience and deserving of the highest consideration, focussing upon the belief that "the objective is a good, all around practical medical man, capable of thinking for himself, resourceful and competent to deal with the principal needs of his patient."

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### The Diet Book

THE proper feeding of the sick, especially when this has to be done at home, is often a problem of no minor degree, for not only are invalids notoriously capricious as to appetite, but special and carefully balanced menus are often desirable if not essential

The purpose of this book, as set forth in the preface, is "To simplify for the general practitioner the labor of detailing to his patients what they may or may not eat, to indicate to the patient himself a dietary suitable, not too monotonous, but at the same time palatable, and to give to the harassed housewife practical suggestions while keeping within the bounds of a prescribed diet"

These purposes are excellently achieved in a concise and practical manner. Special diets are set forth for a week at a time in an easily understandable way and a wealth of recipes and practical information is presented in an attractive manner

The book is well conceived and the plan well carried out. It should be most useful to all concerned

### Breast Feeding†

PERHAPS the common tendency of modern times to wean babies early and avoid, for a variety of reasons, the "strain" of breast feeding, would be less marked if this eminently practical and well written book could be read by every physician and every expectant mother

It can be recommended without reserve as an excellent exposition of a highly important subject

### An Introduction to Pharmacology and Therapeutics

THIS small volume is addressed primarily to the student and intended to provide a short and comprehensive survey of the subject to supplement and not replace lectures. Well written, practical, and concise it well serves the purpose in view

### The Individuality of the Blood‡

THIS book constitutes an invaluable contribution to the literature of a most important subject and will, without doubt, be the standard reference text for years to come

The term "individuality of the blood" signifies those constitutional peculiarities of the blood which enable a distinction between individuals of the same species, a discussion of which, in a strict sense, forms the subject matter of this volume

In conformity with this definition, although much space is naturally given to a discussion of blood groups and their corollaries, the author has not restricted himself to this field but discusses many other phases of the individuality of the blood as related both to clinical and forensic medicine

The present volume is an excellent translation of the French edition of 1929 which has been extensively revised by Professor Lattes and brought into conformity with the latest advances in this field

Following an introductory chapter the subject is discussed under the following subdivisions: Individual Reactions of Normal Blood, The Heritability of the Individuality of the Blood,

\*The Diet Book for Doctor, Patient and Housewife. By Marguerite R. Rea. Cloth 197 pages. Oxford University Press.

†Breast Feeding. By Margaret Emslie. Late Senior Assistant Medical Officer Maternity and Child Welfare County Borough of Croydon etc. Cloth 142 pages 8 plates. Oxford University Press.

\*\*An Introduction to Pharmacology and Therapeutics. By J. A. Gunn. Professor of Pharmacology University of Oxford. Cloth 220 pages. Oxford University Press.

‡The Individuality of the Blood in Biology and in Clinical and Forensic Medicine. By Leone Lattes. Director of The Institute of Forensic Medicine University of Modena. Translated by L. W. H. Bertie. Cloth 413 pages 71 illustrations. Oxford University Press.

The Individuality of the Blood as an Ethnoanthropological Fact, The Individuality of the Blood in Forensic Medicine, and, finally, The Technic of the Individuality Reactions

The section on the heritability of the blood groups has assumed within recent years a definite medico legal importance in questions of parentage and legitimacy and is discussed in extenso, leading to the following conclusions

“1 The hereditary transmission of the blood group is an established fact

“2 This transmission takes place according to Mendel's law and the isoagglutinable properties A and B behave as Mendelian dominants

“3 We may take it as definitely established that the transmission of the blood groups is effected by means of two allelomorphous characters, one being derived from the father and the other from the mother, the possible allelomorphs are three in number (multiple allelomorphs), their combination in pairs giving rise to six genotypical blood groups in man ”

The chapter on forensic applications may be taken as the last word to date upon this subject

As complicated as is this subject, the author's handling of it is remarkably clear, understandable and easily read, a tribute to the skill of the translator. A bibliography, comprising only significant contributions utilized in the text covering 91 pages, testifies to the thoroughness with which the subject has been reviewed

This book may well be considered an epoch making contribution of the highest importance and deserves the widest possible circulation

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Editor WARREN T VAUGHAN, M D  
Richmond, Va

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## *EDITORIAL*

### Focal Infection and the Tonsils

THE acceptance of the tonsils as foci of infection connected with the production of systemic disease has been so extensively studied and so commonly assumed as to render it questionable if, at times, the discovery of infected tonsils has not been made the focus rather than an incident of physical examination

Infected tonsils may, beyond doubt be a primary cause of widely disseminated lesions, but it is also not unlikely that this may be a post hoc propter hoc assumption, and it is, therefore, of some interest to survey observations pertinent to the question

Such a survey, for example is that of Pavlogt and Clowe<sup>1</sup> who reviewed the bacteriology of the tonsils and adenoids in 100 selected cases, 50 per cent being children under eleven years of age

Their purpose was to determine (1) The predominating organism deep in the crypts, (2) whether there was any difference between the organisms in



covered from cases with systemic disease and those in which only local symptoms occurred, and (3) to determine the presence or absence of a carrier state

A conclusion of practical importance was that a surface culture secured by swabbing the tonsil was a reliable index of the predominating bacteriology of the crypts

*Streptococcus hemolyticus* was recovered in 91 cases, 50 per cent being children, whereas the majority of those showing staphylococci as the predominant organism were over twenty-five years old

In 81 per cent both tonsils and adenoids harbored hemolytic streptococci while in ten cases streptococci were found in the tonsils, and staphylococci in the adenoids

In a somewhat similar series<sup>2</sup> of 409 unselected cases, in the majority of which the tonsillar infection was of some standing, the results were as follows

Streptococci, 64 per cent, *Staphylococcus aureus*, 33 per cent, *M. catarrhalis*, 34 per cent, and pneumococci (all types), 54 per cent

These and numerous similar observations suffice to establish the tonsils as potential factors of importance in the production of systemic disease and have led, very naturally to attempts to establish a clear-cut correlation of tonsillar infections with specific diseases

Kaiser<sup>3</sup> from a study of the relation of the tonsils to acute rheumatic fever in childhood involving 439 cases, draws the following conclusions

1 The most susceptible age for the first attack is between eight and fourteen years, the first attack most often developing while the tonsils were still present

2 The incidence of recurring attacks was 10 per cent less when the tonsils were removed than when they were not removed

3 Carditis as a complication was apparently not influenced by tonsillectomy, as was true also of chorea. On the other hand, the association of carditis and chorea was less frequent after tonsillectomy

In view of these findings Kaiser believes that the tonsils are avenues of infection in many cases of rheumatism and that, until more is known of the etiology of this disease, their removal is indicated in the rheumatic and potentially rheumatic child

Lest this should serve as a warrant for promiscuous and indiscriminate tonsillectomy, the same observer<sup>4</sup> from a careful analytical study of twenty-two hundred tonsillectomized children with an equal number of controls, three and ten years after operation, concludes

1 That the real value of removal of tonsils and adenoids cannot be definitely established in a few years, as many apparent early postoperative benefits are not equally evident over a ten-year period

2 That one of the outstanding benefits over a ten-year period is the influence upon the incidence of "sore throats" which show an approximate 25 per cent decrease

3 That the apparent decrease in the incidence of "head colds" and otitis media evident during the three year postoperative period was not maintained over the ten year period while strikingly enough respiratory infections such as laryngitis, bronchitis, and pneumonia were more frequent in the tonsillectomized group

4 That while not striking, there was a definitely perceptible difference in the susceptibility of the tonsillectomized and nontonsillectomized groups to diphtheria and scarlet fever, the difference being in favor of those in whom tonsillectomy had been done

5 That during the ten-year period there was a marked reduction in the incidence of cervical adenitis

6 That in the tonsillectomized group, and especially when tonsillectomy was done early, first attacks of rheumatism were from 30 to 50 per cent less frequent. Where recurring attacks were present no benefit was seen following tonsillectomy

In view of these findings there can be no dissent upon the dicta laid down by Kaiser that tonsillectomy should be regarded as a major operation, that incomplete removal by no means exhibits the same aftermath as complete extirpation in relation to the incidence or prevention of disease, and that the hazards of tonsillectomy must always be considered in the evaluation of the end-results

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R. A. K.

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## NEWS AND NOTES

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### The Maryland Society of Registered Technicians

The Maryland Society of Registered Technicians held their annual meeting in the auxiliary parlors of the Mercy Hospital, Baltimore, Maryland, on April 29, 1933. The following officers were elected: Sister M. J. Wilson, President, V. L. Flannery, Secretary and Treasurer. E. P. Walker and N. E. Behr were appointed members of the board of directors.

This society, which consists of registered laboratory technicians, will meet in the future on the first Saturday of the months of September, November, January, March, and May.

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## CLINICAL AND EXPERIMENTAL

### FURTHER OBSERVATIONS UPON THE COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF AMEBIASIS<sup>2</sup>

AN ANALYSIS OF THE RESULTS OF THE TEST IN ONE THOUSAND INDIVIDUALS

CHARLES F. CRAIG, M.D., M.A., (Hon.) F.A.C.S., F.A.C.P., D.S.M.  
NEW ORLEANS, LA

IN 1927,<sup>1</sup> I demonstrated that the blood serum of individuals infected with *Endamoeba histolytica*, the cause of amebic dysentery, contained complement fixing bodies and in subsequent contributions<sup>2, 3, 4, 5, 6</sup> described the technic and results of a complement fixation test which I devised for use in the diagnosis of this condition. In the present contribution the results obtained with this test as applied to one thousand individuals will be described together with a consideration of the value of the test in diagnosis and in the control of the treatment of amebiasis.

*Historical Summary*—In 1914, Izar,<sup>7</sup> using aqueous extracts of fecal material containing *Endamoeba histolytica* and liver abscess pus obtained from infected kittens as antigens, obtained complement fixation in the blood serum of 5 individuals infected with this parasite and 3 kittens. In 1920, von Hage repeated Izar's work but could not confirm it. In 1921, Scalas, using as an antigen in aqueous extract of mucous flakes from feces containing *Endamoeba histolytica*, obtained positive results in cases of amebic dysentery. No other publications upon the occurrence of complement fixation in amebiasis appeared until mine in 1927.

Since my observations were published the occurrence of complement fixation in amebiasis has been confirmed by several observers, notably Spector, Fernandez, Heathman, and Sherwood and Heathman. Spector<sup>10</sup> using a technic practically identical with mine obtained positive results in cases of amebiasis, Fernandez<sup>11</sup> immunized rabbits by injection of extracts of *Endamoeba histolytica* and demonstrated the presence of complement fixing bodies in

<sup>1</sup>From the Department of Tropical Medicine, School of Medicine, Tulane University of Louisiana.

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the blood serum of these animals, Herthman<sup>12</sup> was also successful in demonstrating complement fixing bodies in the blood serum of rabbits immunized by injections of extracts of *Endamoeba histolytica*, while Sherwood and Herthman<sup>13</sup> obtained complement fixation in the blood serum of cases of amebic dysentery.

*Material*—The observations detailed in this contribution are based upon the results of the complement fixation test upon the blood serum of 1000 individuals suffering from definite symptoms of amebiasis, amebic "carrier" cases having no symptoms of the infection, patients in the wards of a general hospital suffering from other disease conditions than amebiasis, and patients sent to me for examination or seen by me in consultation. In very many of these cases repeated complement fixation tests were made upon the same individual, so that in reality the observations here recorded are based upon the results of at least 2000 complement fixation tests which have been controlled by examinations of the feces for *Endamoeba histolytica*.

*Technic*—The technic employed by me has not differed from that previously described,<sup>14</sup> to which the reader is referred. A human hemolytic system has been used, the sera to be tested have been inactivated by heating at 56° C in the water-bath for one-half hour, and the antigen employed has been an alcoholic extract of forty-eight-hour old cultures of *Endamoeba histolytica* grown upon the Boeck-Dibolav medium. The test was not considered as positive unless a three- or four-plus reaction was obtained on a four-plus scale.

*Results*—This contribution deals only with the complement fixation test in those individuals in whom the results have been checked by an examination of the feces for *Endamoeba histolytica*. To date, 1000 such individuals have been tested, of which 175, or 17.5 per cent gave a positive result and 825, or 82.5 per cent gave a negative result. It should be remembered that the vast majority of the individuals examined were patients in hospital and that many of them were soldiers who had served in localities where amebic infection was common, thus accounting for the comparatively high incidence rate of 17.5 per cent.

Of the 175 individuals giving a positive result, an examination of the feces resulted in the demonstration of the presence of *Endamoeba histolytica* in 157, or 89.7 per cent, while in 18, or 10.2 per cent, the parasite could not be demonstrated in the feces. In justice to the test it should be stated that it was impossible to make more than one or two examinations in the cases negative for the parasite. It is well known that in some cases as many as six examinations must be made of the feces before one is justified in stating that a patient is negative for *Endamoeba histolytica* and that these examinations must be made at intervals of at least two days, as observation has shown that this parasite is frequently impossible of demonstration in the feces at certain times, although it may be present in large numbers at other times. It is very probable that some of these cases would have proved positive for the parasite had a greater number of examinations been made.

Of the 175 individuals giving a positive reaction a record was kept of the occurrence of other intestinal protozoa in 110 cases. Of these, 66 were

infected with *Endamoeba histolytica* alone, 14 with *Endamoeba histolytica* and *Endamoeba coli*, 9 with *Endamoeba histolytica* and *Endolimax nana*, 3 with *Endamoeba histolytica*, *Endolimax nana* and *Endamoeba coli*, 10 with *Endamoeba histolytica* and *Chilomastix mesnili*, 5 with *Endamoeba histolytica* and *Giardia lamblia* and 3 with *Endamoeba histolytica* and *Trichomonas hominis*.

Of the 18 cases in which *Endamoeba histolytica* was not found in the feces, 7 were in individuals having indefinite intestinal symptoms while in 11 a clinical diagnosis of chronic ulcerative colitis had been made.

There were 835 individuals tested who gave a negative reaction, and of these *Endamoeba histolytica* was found in the feces of 12, or 1.4 per cent. Three of these cases were diagnosed acute amebic dysentery, four were diagnosed chronic ulcerative colitis, one, amebic abscess of the liver, three had indefinite intestinal symptomatology and one was symptomless. It is thus evident that not all individuals infected with *Endamoeba histolytica* give a positive reaction with the complement fixation test but the very small percentage that do not, in our experience, is noteworthy.

Many of the negative individuals were infested with other species of intestinal protozoa. Thus, of 676 who gave a negative reaction no less than 220, or 32.5 per cent, were positive for one or more species of intestinal protozoa other than *Endamoeba histolytica*. One hundred, or 14.7 per cent, were infested with *Endamoeba coli*, 64, or 9.9 per cent, with *Endolimax nana*, 3, or 0.4 per cent, with *Endamoeba williamsi*, 26, or 3.8 per cent, with *Chilomastix mesnili*, 16, or 2.3 per cent, with *Trichomonas hominis*, and 11, or 1.6 per cent, with *Giardia lamblia*. There were 14 mixed infections with *Endamoeba coli* and *Endolimax nana*, 6 with *Endamoeba coli* and *Chilomastix mesnili*, 4 with *Endamoeba coli* and *Giardia lamblia*, and 3 with *Endamoeba coli* and *Trichomonas hominis*.

That the infestation of the human intestine with species of amebae other than *Endamoeba histolytica* does not give rise to a positive complement fixation reaction is proved by the fact that of 700 individuals giving a negative reaction, including the 676 mentioned above, no less than 176, or 25.1 per cent, were infested with some other species of intestinal ameba.

Approximately two-thirds of the 825 individuals giving a negative reaction with the complement fixation test were patients in a large general hospital, or from clinics, and were suffering from many acute and chronic disease conditions. The remaining third were normal individuals used as controls. It is thus evident that this test does not give positive results in normal individuals or in those suffering from other disease conditions than amebiasis, with the possible exceptions discussed later.

*The Specificity of the Reaction*—As pure cultures of *Endamoeba histolytica* are not yet available, the antigenic extracts used in this test contain extractives of both this parasite and the bacteria growing in culture with the amebae. Alcoholic extracts of these bacteria, grown upon the Boeck-Drbohlav medium, and prepared in the same manner as the antigen used in the test have been used as controls and in no instance have such extracts given positive

results, so that it is evident that the positive results obtained with the ameba antigen are actually specific and are not due to the bacterial extractives contained in the antigen. That this is true is evidenced by the disappearance of the complement fixation reaction after proper treatment of the amebic infection and the disappearance of *Endamoeba histolytica* from the feces. It has invariably been our experience that the complement fixation reaction disappears after treatment resulting in the disappearance of the amebae from the feces and this is well illustrated in Table I, which gives the time of disappearance of the reaction after treatment in 30 cases in which this could be accurately ascertained.

TABLE I

ILLUSTRATING THE TIME OF DISAPPEARANCE OF THE COMPLEMENT FIXATION TEST FOR *ENDAMOEBIA HISTOLYTICA* AFTER TREATMENT

CASE	RESULT OF TEST BEFORE TREATMENT	FECES EXAMINATION FOR <i>E. HISTOLYTICA</i> BEFORE TREATMENT	RESULT OF TEST AFTER TREATMENT	FECES EXAMINATION FOR <i>E. HISTOLYTICA</i> AFTER TREATMENT	DAYS AFTER CESSATION OF TREATMENT
1	++++	Positive	-	Negative	3
2	++++	Positive	-	Negative	3
3	++++	Positive	-	Negative	4
4	++++	Positive	-	Negative	5
5	++++	Positive	-	Negative	5
6	++++	Positive	-	Negative	6
7	+++	Positive	-	Negative	7
8	++++	Positive	-	Negative	9
9	++++	Positive	-	Negative	11
10	+++	Positive	-	Negative	12
11	++++	Positive	-	Negative	14
12	++++	Positive	-	Negative	14
13	++++	Positive	-	Negative	14
14	++++	Positive	-	Negative	14
15	++++	Positive	-	Negative	14
16	++++	Positive	-	Negative	14
17	++++	Positive	-	Negative	14
18	++++	Positive	-	Negative	14
19	++++	Positive	-	Negative	14
20	++++	Positive	-	Negative	14
21	++++	Positive	-	Negative	14
22	++++	Positive	-	Negative	14
23	++++	Positive	-	Negative	14
24	++++	Positive	-	Negative	14
25	++++	Positive	-	Negative	14
26	++++	Positive	-	Negative	18
27	++++	Positive	-	Negative	21
28	++++	Positive	-	Negative	21
29	++++	Positive	-	Negative	21
30	++++	Positive	-	Negative	28

Upon reference to Table I it will be noted that the reaction disappeared in 2 cases, three days after the cessation of treatment, in 1 case four days afterward, in 2 cases, five days afterward, in 1 case each, six, seven, nine, eleven, twelve, eighteen, and twenty-eight days after cessation of treatment respectively, in 15 cases, fourteen days afterward, and in 3 cases, twenty-one days after cessation of treatment. It is evident that the time of disappearance of the reaction after treatment varies considerably in different individuals as would be expected, but that in the vast majority of the cases, as shown

in Table I, the reaction had disappeared by the end of the second week after the cessation of specific treatment and the disappearance of *Endamoeba histolytica* from the stools. Thus of the 30 cases studied, the reaction had disappeared within fourteen days in no less than 25 or 86.6 per cent, while in the remainder it had disappeared by the twenty eighth day after the cessation of treatment. The disappearance of the reaction after treatment resulting in the elimination of *Endamoeba histolytica* demonstrates, in my opinion, the specificity of this complement fixation test for infection with *Endamoeba histolytica*. It may be stated that similar results are obtained in experimental amebiasis in dogs, as recently reported by me.<sup>12</sup>

The only conditions in which apparently false positive reactions have been obtained with this test are syphilis and chronic ulcerative colitis. Of 110 individuals giving a positive complement fixation test for infection with *Endamoeba histolytica*, 15 or 13.6 per cent, also gave a positive Wassermann and Kahn test, while of 676 individuals giving a negative reaction for *Endamoeba histolytica*, 56, or 8.2 per cent, gave a positive Wassermann or Kahn test. Of the 15 individuals giving a positive complement fixation test for *Endamoeba histolytica* as well as a positive Wassermann and Kahn test, 9 failed to show *Endamoeba histolytica* in the feces. Because of the greater number of cases giving a positive reaction with all three tests and the failure to find *Endamoeba histolytica* in over half of such cases, I have stated<sup>10</sup> that "in rare instances, patients suffering from syphilis may give a positive result with this test." However, further observations have convinced me that this is not true and that syphilis does not give a reaction with the complement fixation test for amebiasis. This conclusion is based upon the observation that in properly treated cases positive with the three tests, the complement fixation reaction disappears after the elimination of *Endamoeba histolytica* while the Wassermann and Kahn reactions persist, and that the apparent excess of positive Wassermann and Kahn reactions in individuals giving a positive complement fixation test for amebiasis disappears as a larger number of individuals infected with this parasite have been tested. At the present time it is not believed that syphilis gives a positive result with this test unless there is a coincident infection with *Endamoeba histolytica*.

The question of the occurrence of a positive complement fixation reaction with this test in certain cases of chronic ulcerative colitis is an interesting and important one. In 1932, Kiefer,<sup>17</sup> of the Lahey Clinic, reported the results of this test in 19 cases diagnosed as chronic ulcerative colitis of which 15 gave a positive result. Of the 15 positive cases *Endamoeba histolytica* could be demonstrated in only 3 cases. The complement fixation tests in this series of cases were made for Dr. Kiefer by me, while the stool examinations were made in Boston by competent parasitologists. In his paper, Kiefer states that cases of diarrhea caused by functional disturbance, also tested by me, gave consistently negative results, and that the large percentage of positive results in chronic ulcerative colitis remains unexplained and suggests some relationship between this condition and amebic infection. He suggested that chronic ulcerative colitis may be "a progonic infection of the colon superimposed upon an original amebic ulceration."

In my personal experience, exclusive of the tests made for Dr. Kiefer, there have been eleven cases diagnosed as chronic ulcerative colitis which have given a positive reaction with the complement fixation test in which the examination of the feces for *Endamoeba histolytica* resulted negatively. In all of these cases it was impossible to make more than one or two examinations of the feces, so that it is impossible to be sure that the parasite was really absent, for in many other cases diagnosed chronic ulcerative colitis in which the reaction was positive, repeated examinations had to be made in order to demonstrate the parasite. In several such cases as many as six to eight examinations of the feces were made before *Endamoeba histolytica* was found and, even then, in small numbers. In some cases daily examinations were made for over a week and the parasite found at the last examination, so that unless at least six examinations are made upon different days it is impossible to be sure that *Endamoeba histolytica* is not present. In view of this experience I am convinced that this test does not give a positive result in cases of chronic ulcerative colitis unless *Endamoeba histolytica* is present. This conclusion is further justified by the results of antiamebic treatment in these cases, for Kiefer states that treatment with chiniofon of the cases of chronic ulcerative colitis giving a positive reaction with the complement fixation test, but negative for *Endamoeba histolytica*, resulted in the disappearance of the symptoms in 4 cases with no return for from ten to fourteen months, a definite improvement in 5 cases, while in the remaining the treatment had not been completed and concludes that antiamebic treatment should be tried in all cases of chronic ulcerative colitis. My experience agrees with that of Kiefer as regards the specific effect of antiamebic treatment in cases of ulcerative colitis giving a positive complement fixation test, but negative for *Endamoeba histolytica*, for all cases so treated have been benefited or apparently cured.

The theory of Kiefer that chronic ulcerative colitis may be a pyogenic infection engrafted upon an original amebic infection is worthy of serious consideration and study. It would explain the occurrence of a positive complement fixation test in some cases of this condition in which the parasite cannot be demonstrated in the feces, for in such cases there may be a localization of the organisms in the tissues of the intestine with few or none occurring in the lumen of the gut, the conditions there being unfavorable for the propagation of the amebae, owing to the presence of the pyogenic infection. In time, the amebic infection in the tissues would disappear and in such cases the complement fixation reaction will result negatively, as it does in many cases diagnosed as chronic ulcerative colitis, in my experience. At the present time it is believed that the occurrence of a positive reaction with the complement fixation test for amebiasis in certain cases of chronic ulcerative colitis indicates the presence in the tissues of the colon of *Endamoeba histolytica* and that when this parasite is absent positive reactions will not be obtained in this condition.



With the exception of the conditions discussed there is no evidence that the complement fixation test for amebiasis gives positive results in either normal individuals or those suffering from other diseases.

*The Value of the Test in Diagnosis*—Continued observations with this test have convinced me of its value in the diagnosis of amebiasis. Where the services of a trained protozoologist are available, and it is possible to make repeated microscopic examinations of the feces in suspected cases, this test would be of comparatively little help in diagnosis, as I am convinced that every case of intestinal infection with *Endamoeba histolytica* can be diagnosed by microscopic examinations of the feces, provided repeated examinations are made, if necessary. However, the test has proved of value in my hands in the discovery of cases of amebic abscess of the liver in patients whose feces were negative for the parasite in four instances, and in several other cases the routine use of the test has resulted in the discovery of liver abscesses due to *Endamoeba histolytica*, in which subsequent fecal examinations demonstrated the presence of the parasite, although no symptoms of amebic infection of the intestine were present. The routine application of the test has also given positive results in scores of cases where an amebic infection was not suspected and where the symptoms were atypical or so slight as to be overlooked by the clinician. The discovery and proper treatment of such infections is of the greatest importance from both a therapeutic and prophylactic standpoint. I<sup>2</sup> have recently reviewed the evidence demonstrating that marked ulcerative lesions due to *Endamoeba histolytica* may exist in the intestine of individuals who have shown no clinical evidence of amebic infection, so that absence of symptoms does not prove that amebic infection is absent or that definite pathologic lesions of the intestine may not exist, and the complement fixation test for amebiasis should prove of the greatest service as an aid in the recognition of such cases of latent amebiasis.

The use of the test as a *control of treatment* should also prove of definite value. As already stated, proper treatment followed by the disappearance of *Endamoeba histolytica* from the feces, is also followed by the disappearance of the positive complement fixation reaction, and, in cases which have relapsed, by a return of the positive reaction. It is thus possible to determine the effect of treatment with any particular drug and the relative efficiency of various methods of medication by repeated complement fixation tests. In some cases in which a relapse occurred a return of the positive complement fixation reaction has been noted even before the amebae could be demonstrated in the feces, thus indicating a multiplication of the organisms in the tissues of the intestine sufficient to produce such a reaction before they become numerous enough in the lumen of the bowel to be demonstrable in the feces.

At the present time the practical application of the test is limited by the difficulty of preparing an efficient antigen, and the small amounts that can be obtained from large numbers of cultures of *Endamoeba histolytica*. It is hoped that, in the near future, improved methods of preparing antigens will make it possible for the test to be more generally used in clinical laboratories than it is at present.

## CONCLUSIONS

1 Specific complement fixing bodies may be demonstrated in the blood serum of individuals infected with *Endamoeba histolytica* when alcoholic extracts of cultures of this parasite are used as antigens

2 Normal individuals or those suffering from other infections or diseases do not give a positive complement fixation reaction with such antigens unless there is a coincident infection with *Endamoeba histolytica*

3 The specific complement fixing bodies disappear from the blood serum following antiamebic treatment and the disappearance of *Endamoeba histolytica* from the feces

4 In relapsing cases of amebiasis the complement fixation test, which has been negative during the interval of apparent freedom from *Endamoeba histolytica*, again becomes positive, in rare instances even before the parasite is again demonstrable in the feces. Thus, a negative reaction unless repeatedly so for several weeks, does not prove the absence of amebic infection or that antiamebic treatment has resulted in cure

5 The time of disappearance of the positive complement fixation reaction after treatment resulting in the disappearance of *Endamoeba histolytica* from the feces has varied, in this series of observations, between three and twenty-eight days. In 86.6 per cent of the cases studied the reaction disappeared within fourteen days after the cessation of antiamebic treatment, and in 96.6 per cent within twenty-one days after cessation of treatment resulting in the disappearance of the parasite from the feces

6 Individuals infested with other species of amebae, or with the intestinal flagellates, do not give a positive complement fixation with this test unless *Endamoeba histolytica* is also present

7 The complement fixation reaction occurs in all stages of amebiasis but the strongest positive results are usually obtained in symptomless "carriers" or in those presenting mild symptoms of infection with *Endamoeba histolytica*. In several cases of very acute amebic colitis the reaction has been doubtful or negative, although in most severe cases the reaction has been positive

8 The test has proved of value in the diagnosis of cases of amebic abscess of the liver unaccompanied by intestinal symptoms, in the diagnosis of apparently healthy "carriers" of *Endamoeba histolytica* and of those presenting atypical or mild symptoms of infection, and in the control of antiamebic treatment

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## RESULTS OF TREATMENT IN RHEUMATOID ARTHRITIS WITH REFERENCE TO FOCI OF INFECTION AND STREPTOCOCCUS VACCINE<sup>2,†</sup>

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IN RECENT years two forms of therapy have arrested the attention of physicians engaged in the study of rheumatoid arthritis, namely, the removal of foci of infection and the administration of streptococcus vaccine.

As far back as twenty years ago, Billings<sup>1</sup> pointed out the relationship of foci of infection to chronic arthritis. Since that time, certain investigators have enthusiastically advocated the radical removal of such foci in arthritic patients, some have been conservative regarding this procedure, while others have been frankly skeptical. Even today, there seems to be no unanimity of opinion as to the value of removing infected foci in the treatment of this disease.

The other form of therapy which we are considering in this paper, the administration of streptococcus vaccines, has recently enjoyed considerable popularity. Several different types of streptococci have been employed in the preparation of these vaccines, and two methods of administration, the subcutaneous and the intravenous have been adopted. Regardless of the

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<sup>†</sup>Rheumatoid arthritis is considered synonymous with chronic infectious arthritis and atrophic arthritis.

type of streptococcus used, or the method of administration, the therapeutic results have generally been reported as favorable<sup>2</sup>

#### THE PRESENT STUDY

The present study was undertaken in the hope of crystallizing our own impression as to the relative value of removing different foci of infection in patients with rheumatoid arthritis, as well as to determine the merits of streptococcus vaccine therapy. The patients included in this report were ambulatory and were treated in the Arthritis Department of the Cornell Clinic between 1923 and 1932. As attendance at this clinic requires payment for services, the patients were of moderate means, lived in favorable environments, and were intelligent and cooperative, all resided in the metropolitan area of New York City, and when able they were permitted to pursue their usual occupations. In order to avoid confusion, individuals having more than one type of focus of infection removed were not included in this study. When patients were treated with vaccine only, either foci of infection had not been discovered or had been previously removed.

Forms of treatment supplementary to removal of foci of infection and administration of vaccine were limited to a minimum. As the average patient with rheumatoid arthritis is under-nourished, a generous unrestricted diet, high in vitamin content, was usually recommended. The markedly overweight were given a diet limited in carbohydrates, but they comprised a small proportion of our series. Aspirin and sodium salicylate were frequently prescribed for the relief of pain, and sometimes patients were advised to apply heat to the affected joints, by means of an electric pad, infra-red lamp, or hot compresses. Cod liver oil was given in some cases.

In our experience, patients with well developed rheumatoid arthritis are seldom cured of the disease, if the word *cured* implies complete and permanent relief from all pain, swelling, and stiffness. The few who were discharged as *cured* have usually, at some later period, developed at least vague and transitory symptoms and sometimes suffered complete relapse. For these reasons, we have not classified any of our cases as *cured*, being content to use the word *improved*. Considerable care has been exercised at all times not to confuse temporary changes due to climatic or other varying conditions with more lasting ones. No patient has been included who has not been under observation for at least two months, and many have been followed for several years.

For convenience of study the patients were divided into three groups according to clinical severity. The first group comprises those who complained of pains in the joints, but who failed to show any evidence of arthritis at the time of examination other than tenderness and stiffness of the joints. The second group includes those who presented the typical picture of well developed rheumatoid arthritis with characteristic periarticular swelling of the joints, usually including fusiform fingers. The third group is similar to the second, but comprises patients who were in a more advanced stage of the disease, deformities such as ulnar deviation, and partial or complete ankylosis of

the joints characterize this group. In the tables these three groups have been represented by the symbols - and - respectively.

#### VACCINES

*Preparation of Vaccines*—The various vaccines used in this study were similarly prepared. When several strains were to be included in one vaccine each was treated separately during the early stages of the process. Sufficient growth was obtained by cultivation in beef-heart infusion broth for eighteen to twenty-four hours. After centrifuging the culture, the supernatant broth was discarded and the sediment washed twice in saline. The bacteria were then suspended in physiologic saline at a concentration of one billion organisms per cubic centimeter and bottled, 0.15 per cent trikresol being added for killing and preserving. After shaking, the bottles were allowed to stand in an inverted position for forty-eight hours. Tests for sterility were then made by culturing the vaccine in blood broth and blood agar.

*"Typical Strain" Vaccine*—In a previous article we, in collaboration with Cecil,<sup>3</sup> reported the isolation of streptococci from the blood of a high percentage of patients with rheumatoid arthritis. Of the streptococcus strains thus recovered 83.3 per cent were similar both culturally and biologically, and were designated as "typical strains." On laboratory mediums, these strains presented the appearance of attenuated hemolytic streptococci. In later papers,<sup>4</sup> it was shown that the serums of a high percentage of patients with well developed rheumatoid arthritis gave strong specific agglutination reactions with "typical strain" streptococci. Because of the apparent etiologic relationships of these organisms to rheumatoid arthritis, they were considered the most suitable for vaccine therapy, and ten of them were used in the preparation of the so called "typical strain" vaccine. The vaccines were made in large quantities about every three months, the same strains being used each time. Stock cultures of these organisms were maintained in the frozen and dried state as well as in blood broth and blood agar mediums.

*Other Streptococcus Vaccines*—Autogenous vaccines were prepared as soon as possible after the streptococci had been isolated, in sufficient quantity to last a patient throughout the course of his treatment. Polyvalent streptococcus vaccines were made from organisms recovered from a wide variety of sources and varied from time to time.

*Subcutaneous Administration*—All vaccines for subcutaneous use contained one billion organisms per cubic centimeter. Injections were given at weekly intervals beginning with 0.1 c.c. and increasing the amount each time until a maximum of 1 c.c. was reached. The treatment was continued at this final level throughout the remainder of the course. Mild local reactions, as indicated by swelling, tenderness, and pain at the site of injection, frequently occurred but severe local reactions, and generalized reactions as indicated by fever and malaise were seldom noted. If such reactions did develop, the amount of vaccine was increased more gradually.

*Intravenous Administration*—The vaccine for intravenous use was prepared merely by diluting the subcutaneous vaccine with physiologic saline.

Injections were given at weekly intervals, beginning with 50,000 organisms, and increasing the amount at each succeeding inoculation, according to varying rates. For a certain number of patients, the dosage was increased by about 50,000 organisms each time, a single injection never exceeding one million organisms. For others, the rate of increasing the amount injected was more rapid, and at about the tenth injection approximately ten million organisms were being given. Subsequent doses for these patients were then maintained at the ten million level. The dosage for twenty patients in the group receiving "typical strain" intravenous vaccine only and for a few in the group receiving both subcutaneous and intravenous injections was increased by the simple process of doubling the amount of the previous injection until a febrile reaction occurred. Subsequent injections were regulated to a point where the patient developed no more than a mild generalized reaction. Febrile reactions were generally elicited when a patient received an injection containing between one hundred and five hundred million organisms although one patient who was given a billion organisms at one time had no reaction whatever. Regardless of whether the injections were small or large, the percentage of improvement was similar and, therefore, all of these cases are classified together.

#### RESULTS OF TONSILLECTOMY

It is often a matter of considerable difficulty to determine whether any particular pair of tonsils is or is not diseased. For this reason, some physicians recommend tonsillectomy for all patients suffering from rheumatoid arthritis, providing their general physical condition is satisfactory. In the present series, however, this policy was not adopted. Tonsils were removed only when it was the opinion of the Nose and Throat Department that they were infected. In making a diagnosis of diseased tonsils, emphasis was placed on a history of previous attacks of sore throat, tonsillitis, and peritonsillar abscess, the size and fixity of the tonsils, the color of the anterior pillars, and the possibility of expressing exudate from the crypts were also taken into consideration.

Altogether, 103 patients were subjected to tonsillectomy and of these, 60, or 58.3 per cent, were improved (Table I). In some patients, the change was immediate, in others, it was gradual, extending over a period of weeks or months, while there were a few cases in which the joint symptoms were temporarily increased, although lasting improvement was eventually noted. Among the unimproved was a small number whose arthritis was apparently worse as a result of the operation. Twenty patients received no therapy other than tonsillectomy. The remaining eighty-three were given in addition some form of streptococcus vaccine, although they in no wise represented a group unimproved by tonsillectomy, the injections being frequently started either before this operation or shortly afterward. In some cases the injections were subcutaneous, in others, intravenous, while certain of the patients

TABLE I  
RESULTS OF TONSILLECTOMY

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Tonsillectomy only	20	12	60.0
Tonsillectomy and "typical strain" vaccine	57	31	54.4
Tonsillectomy and other streptococcus vaccines	26	17	65.4
	83	48	57.8
Total	103	60	58.3

received both. The method of preparation of the vaccine, as well as the manner of increasing the dosage was similar to that used with patients who received vaccine treatment alone. The average number of injections for the patients in this group was 21.2, although there was considerable variation in the individual dosage. Fifty-seven were given "typical strain" vaccine, and of these, 31, or 54.4 per cent, were improved. Twenty-six received various other kinds of streptococcus vaccine, such as autogenous vaccines prepared from hemolytic, green producing and indifferent streptococci, alone or combined. Of these 26 cases, 17, or 65.4 per cent, were improved, but, on careful analysis, no particular merit could be found for any of the types of vaccine employed, and they were, therefore, classified together.

Although the number of patients having tonsillectomy only was small, it was interesting to note that they showed as high a percentage of improvement as those having both tonsillectomy and streptococcus vaccine.

In Table II the cases are arranged according to the severity of the disease. The group having mild arthritis showed an improvement of 66.7 per

TABLE II  
RESULTS OF TONSILLECTOMY ACCORDING TO SEVERITY OF DISEASE

SEVERITY OF DISEASE	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
+	27	18	66.7
++	51	30	58.8
+++	25	12	48.0
Total	103	60	58.3

cent, that with well developed signs, 58.8 per cent, and the still more advanced, 48.0 per cent. As would be expected, the mild cases benefited more from tonsillectomy than the severe ones.

Age also played an important rôle in the patient's chances toward recovery (Table III). Of 58 patients forty years of age or under, 39, or 67.2 per cent, were improved, compared to 45 over forty years of age with improvement in only 21, or 46.7 per cent.

The duration of the disease, on the contrary, was apparently an unimportant factor (Table IV). Of those having the disease one year or less, 59.2 per cent were improved, of those having it for more than a year, 57.4 per cent were improved, remarkably similar figures.

## RESULTS OF REMOVING ABSCESSSED TEETH

In studying the effect on arthritis of the removal of diseased teeth it was considered advisable to include only patients whose teeth showed definite evidence of abscess formation. The diagnosis was largely made from the study of x-rays and was frequently confirmed by examination of the extracted teeth. Simple pyorrhea and dental caries were not included, no matter how profound.

TABLE III

## RESULTS OF TONSILLECTOMY ACCORDING TO AGE

AGE	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Forty years of age or under	75	39	67.2
Over 40 years of age	45	21	46.7
Total	103	60	58.3

TABLE IV

## RESULTS OF TONSILLECTOMY ACCORDING TO DURATION OF DISEASE

DURATION	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
One year or less	19	29	59.2
More than one year	34	31	57.4
Total	103	60	58.3

Altogether, 35 patients were studied and of these, 24 or 68.6 per cent, were improved (Table V). Five of the patients received no other therapy, while 30 were given in addition streptococcus vaccines of types similar to those used in conjunction with tonsillectomy. In the results of vaccine ther-

TABLE V

## RESULTS OF ABSCESSSED TEETH EXTRACTION

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Teeth only	5	5	
Teeth and streptococcus vaccine	30	19	63.3
Total	35	24	68.6

apy alone, reported later in this paper, the percentage of improvement was low, and it seems reasonable, therefore, to attribute the high percentage of improvement chiefly to the removal of the dental foci. The average number of injections for patients in this group was 19.1.

The number of patients having teeth extraction is too small to warrant a detailed analytical study. They were somewhat older than those subjected to tonsillectomy, 11 being forty years of age or under, while 24 were over forty. The milder cases showed a greater tendency toward improvement than did the more severe.



## OTHER FOCI OF INFECTION

Next to diseased tonsils and teeth, infected sinuses appeared most prominently as a focus of infection in patients with rheumatoid arthritis. Because the treatment of infected sinuses is generally so unsatisfactory, it was considered advisable to omit this form of therapy from the present study. In the Cornell Clinic, other foci of infection were seldom noted.

## RESULTS OF VACCINE THERAPY ALONE

Altogether 194 patients were treated with vaccine therapy only, and of these, 69, or 35.6 per cent, showed improvement (Table VI). Of the 87 patients receiving "typical strain" vaccine subcutaneously 32, or 36.8 per cent, showed improvement, of the 36 receiving the same vaccine intravenously 13 or 36.1 per cent, showed improvement, while of the 48 receiving it both subcutaneously and intravenously 14, or 29.2 per cent, showed improvement. Twenty-three others were given various kinds of streptococcus vaccines with improvement in 10, or 43.5 per cent.

The average number of injections for patients in each group is indicated in Table VI.

Regardless of the type of vaccine or the method of administration remarkably similar results were recorded.

TABLE VI  
RESULTS OF STREPTOCOCCUS VACCINE

	NUMBER OF CASES	AVERAGE NUMBER INJECTIONS	NUMBER IMPROVED	PER CENT IMPROVED
"Typical Strain" vaccine, subcutaneous only	87	34.9	32	36.8
"Typical Strain" vaccine, intravenous only	36	15.7	13	36.1
"Typical Strain" vaccine, subcutaneous and intravenous	48	24.1	14	29.2
Other forms of streptococcus vaccine (subcutaneous only)	23	18.1	10	43.5
Total	194	26.7	69	35.6

TABLE VII  
RESULTS OF STREPTOCOCCUS VACCINE ACCORDING TO SEVERITY OF DISEASE

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
+	67	22	32.8
++	94	38	40.4
+++	33	9	27.3
Total	194	69	35.6

In Table VII, the results of treatment with vaccine only are tabulated according to the severity of the disease. The mild cases showed an improvement of 32.8 per cent, the well-developed, 40.4 per cent, and the severe 27.3 per cent results from which little can be deducted.

In Table VIII the patients treated with streptococcus vaccine only are grouped according to age. Those forty years of age or under showed an improvement of 44.3 per cent, while those over forty showed only 29.6 per cent improvement.

Table IX reports the results in the same group of patients as in Table VIII, but viewed from the standpoint of the duration of the disease. Of 77 patients having the disease for one year or less, 46.8 per cent improved, whereas of 117 having the disease for more than one year, only 28.2 per cent were improved.

The results shown in Tables VIII and IX are extremely difficult to interpret. The figures may indicate that young patients and those having the disease for only a short time received some benefit from streptococcus vaccine therapy, on the other hand, they may only indicate that such patients have a somewhat greater tendency toward spontaneous self-limitation of the disease. To us the latter interpretation seems more probable.

TABLE VIII  
RESULTS OF STREPTOCOCCUS VACCINE ACCORDING TO AGE

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
Forty years of age or under	79	35	44.3
Over 40 years of age	115	34	29.6

TABLE IX  
RESULTS OF STREPTOCOCCUS VACCINE ACCORDING TO DURATION OF DISEASE

	NUMBER OF CASES	NUMBER IMPROVED	PER CENT IMPROVED
One year or less	77	36	46.8
More than one year	117	33	28.2

#### SUMMARY AND DISCUSSION

This paper is a presentation of the results of the treatment of 332 patients with rheumatoid arthritis by removal of foci of infection and with the administration of certain streptococcus vaccines.

One hundred and three patients were treated by removing diseased tonsils and 60 (58.3 per cent) showed subsequent improvement of their arthritic condition. Twenty of these had no other form of therapy, while 83 were given in addition streptococcus vaccine. No higher rate of improvement was noted in patients having vaccine and tonsillectomy combined than in those having tonsillectomy alone.

As would be expected, the results in the tonsillectomy group were best in young patients and those having mild arthritis, the duration of the disease appeared to be an unimportant factor in the chances of recovery.

Thirty-five patients had abscessed teeth removed, of which 24 (68.6 per cent) showed improvement. Thirty of the thirty-five received streptococcus

vaccine therapy also. Taking into consideration the low percentage of improvement in patients having vaccine therapy only, it seems reasonable to assume that the high percentage of improvement in patients with dental extraction and vaccine therapy can be largely attributed to the removal of the abscessed teeth per se.

One hundred and ninety-four patients were treated with vaccine therapy alone. Of these only 69 (35.6 per cent) showed improvement. The results were remarkably similar, regardless of the type of vaccine used, or of the method of administration.

Throughout this study, complete and permanent cure was an exceedingly rare occurrence. The removal of diseased tonsils and abscessed teeth, however, favorably modified the course of the disease in a high percentage of cases.

Although our policy has been to advise removal of foci only when there was strong evidence that they were a source of infection, we feel that perhaps this strict ruling has in some instances permitted the retention of foci responsible for the arthritic condition.

In estimating the results of vaccine therapy in rheumatoid arthritis, one is impressed with the fact that we have no measuring rod for accurately determining the severity of the disease in any individual patient. The erythrocytic sedimentation test offers possibilities for such a determination, but further work must be done in this field.

That vaccine inoculation undoubtedly has a considerable psychological effect on the patient should be taken into consideration in estimating the results of this type of therapy.

In regard to the series of cases here reported, it is, of course, admitted that the employment of other forms of streptococci or other methods of preparing vaccine might have been attended with more satisfactory results.

Although we do not know the percentage of patients who would have improved spontaneously, our results with vaccine therapy were extremely disappointing, as the low percentage of improvement may well represent the natural tendency of some patients to improve regardless of treatment, rather than to any inherent value in streptococcus vaccine therapy.

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## YEAST-LIKE FUNGI\*

### DIFFERENTIAL CHARACTERISTICS AND CASE REPORTS

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THE literature shows a wide disagreement among investigators as to the cultural, biochemical, and morphologic characteristics of yeast-like fungi parasitic to man. Robins<sup>1</sup> first studied the morphologic characteristics of the thrush fungus and, not recognizing the similarity of this fungus to *M. candida* described previously by Bonoiden,<sup>2</sup> he named it *oidium albicans*, *oidium* being the term applied by Link, 1809, to the fungus of grapevine mildew and later by Fresenius, 1850, to name a fungus isolated from sour milk. The name of the thrush fungus has changed several times since its recognition. Reess,<sup>3</sup> 1870, reported the presence of asci and called it *saccharomyces albicans*. After the advent of pure cultural methods, Zopf<sup>4</sup> found no asci in the thrush fungus, recognized its identity with yeast-like fungi of the genus *monilia* and renamed the organism *Monilia albicans*. The nomenclature was again changed by Vuillemin, 1898, when he reported the formation of asci. He named it *Endomyces albicans*. Since then the work of Zopf has been substantiated and the organism has retained the name given by him *M. albicans*.

Multiplicity of species among the cultures isolated from cases of thrush was recognized by Plaut<sup>5</sup> 1887. He studied cultures of what he designated as "Thrush Fungus" and *M. candida* and came to the conclusion that from the standpoint of morphology and pathogenicity they are identical. Multiplicity was also recognized by Hefft,<sup>7</sup> 1895. He observed that organisms of this group were associated with disease processes involving the mucosa of the vagina. He described 26 cases of vaginitis due to a yeast-like fungus and identified the organism from 4 of the cases as *M. candida* and from 16 as *M. albicans*. Craik,<sup>8</sup> 1913, also recognized the multiplicity of species in this genus and described both *M. candida* and *M. albicans*.

The term "blastomyces" was first used by Busse<sup>9</sup> in Germany and Gilchrist in this country to designate a yeast-like pathogenic fungus the exact

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identity of which was puzzling. Since then the term blastomycosis has come to cover all disease processes with which a yeast like organism is associated, and the term blastomyces to designate such organisms as saccharomyces, cryptococci, monilia, oidium, torula, and endomyces.

Recently there has been an effort to classify these organisms more strictly. For this purpose sugar fermentations, litmus milk, and gelatin reactions have been used. The variable results from these studies have caused considerable confusion and the consequence has been a great increase in the multiplicity of species especially in the monilia group. Castellani identified up to 1920 forty-two different species of monilia. Later, disregarding acid and using only gas production in certain sugars together with gelatin and litmus milk reactions, he reduced this number to 28.

Following this work the literature abounds with reports of yeast-like fungi associated with various disease conditions, bronchitis, pneumonia, asthma, thrush, vaginitis, etc. The organisms reported are given a variety of names. From this review, it is obvious that the literature is confusing as to the classification of the yeast-like fungi pathogenic for man and particularly as it applies to monilia. All of the investigators have reported a variability of these organisms in their cultural and biochemical characteristics. This variability has been accepted as due to the variability of the organisms under identical environmental circumstances.

Because of this confusion and because we saw frequently in sputum, which was being examined for tubercle bacilli, yeast-like cells and mycelium we became interested in their identification and classification. In 1928,<sup>10</sup> one of us, Stovall, reported 18 cases of primary pulmonary infection with yeast-like fungi. No other name was given to the organisms isolated, because we were confused as to their identity. Since then we have isolated 200 cultures from cases of bronchitis, pneumonia, asthma, bronchiectasis, thrush, and vaginitis. In 1929<sup>11</sup> we reported a cultural study of 40 of these strains. We did not encounter the variability that has been so frequently reported, but to the contrary when it was possible to repeatedly establish the same environment as to composition of media, temperature, time of incubation and to observe the results at the same time in the incubation period, we found that the individual organisms and those in the same species showed remarkably constant morphologic, cultural and biochemical characteristics. Among these cultures we encountered only three species of monilia.

We were impressed with the constancy of the results and also with the limited number of species encountered. We thought that possibly the latter experience was due to the locality and therefore decided to study cultures of monilia secured from the American type culture collection. We selected certain cultures because of the investigators who first studied them and also because of the locality in which the investigator was working at the time the organism was isolated. In this study<sup>12</sup> we found that all of the cultures of monilia received from the American type culture collection could be classified with one of the species we had identified among our cultures, and also that when submitted to our method of study, they were as constant in their behavior to the various medias as our own cultures.

We have now completed the study of 250 cultures isolated by one of us (Bubolz) from cases of bronchitis, pneumonia, asthma, thrush, vaginitis, and generalized infections. We find that all of the monilias behave in a constant manner and that among all of these cultures of monilia there exist only three species.

This paper is for the purpose of a detailed description of the methods we have employed and also to record the different varieties of organisms which we have encountered together with the clinical characteristics associated with these infections.

#### MATERIAL AND METHODS

The 250 organisms used in the study were isolated from patients suffering from bronchitis, asthma, pneumonia, thrush and vaginitis, and cultures obtained from the American type culture collection.

*Microscopic*—The sputum was examined first by making moist preparation for microscopic study. The material was selected for these preparations



Fig 1—Monilia in sputum

by pouring the sputum into a sterile Petri dish and, with the back of the table top for a background, picking the small flakes of grayish white material. These small flakes are composed of tangled threads of mycelium, epithelial cells and fibrin. They are loosely held together and as a rule no sodium hydroxide is needed to soften them. In some instances when the material has been too tough and hard to satisfactorily press out under a coverslip, we have treated it with a 20 per cent sodium hydroxide solution. This method of examination reveals in diseases associated with the presence of certain yeast-like fungi, monilia and endomyces many tangled threads of mycelium and many yeast-like cells some of which are seen to bud (Fig 1). In other varieties of yeast-like fungi, oidia and torula, no mycelium is seen but the yeast-like cells are present (Fig 2, A and B). Occasionally this examination will fail to reveal the organisms which will show up in the cultures. It is important, however, to demonstrate the organism in the moist preparation since it lends considerable emphasis to the interpretation of the finding. Their presence in large numbers in the sputum particularly in the

grayish-white particles, certainly associates them with the exudate and secretion coughed up from the bronchi and rules out mouth contamination. This is particularly true if no mouth lesions can be demonstrated. The interpretation of positive cultural results and negative moist preparations requires repeated study in the laboratory as well as at the bedside.

In the case of thrush or vaginitis, swabs are used to collect the exudate, and from these glass slides, smears and cultures are made. The smears are stained by Gram's method and reveal either yeast-like cells or mycelium or both.

*Isolation*—This can be accomplished from 1 per cent glucose broth cultures or directly from sputum or inflammatory exudates. The culture or exudate is plated on malt and plain agar. The plates are incubated at 37° C. In our experience any of these fungi except oidium will show on malt agar, after forty-eight hours incubation colonies measuring from 2 to 5 cm. Oidium

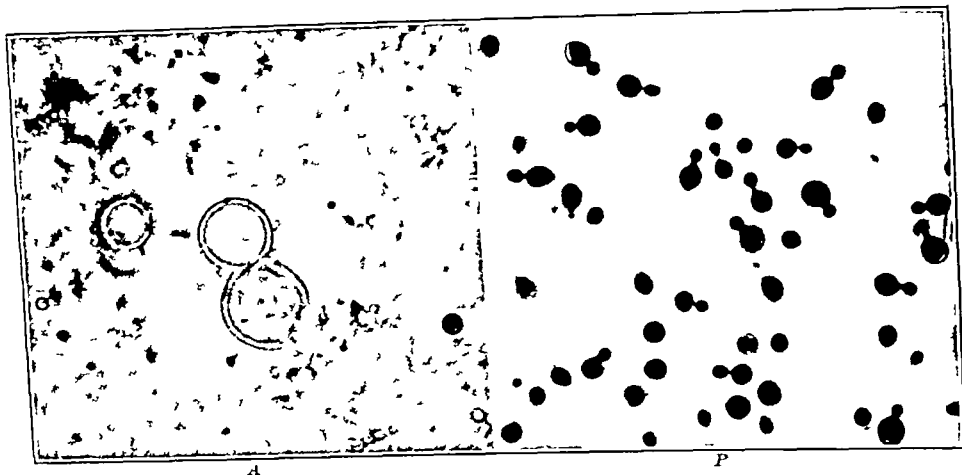


Fig 2—A, Oidia (blastomycetes) in sputum B, Torula forty-eight hour broth culture

does not develop so rapidly on malt agar but develops similar sized colonies on plain agar in a week to ten days.

*Pure Culture Study*—This is made for the further identification and classification of the organisms. For this purpose we have studied morphology, method of reproduction, colony morphology on malt agar plates, fermentation of various carbohydrates, gelatin liquefaction, and reactions in milk.

On malt agar plates the monilia show characteristic colony morphology for the three species at the end of forty-eight hours incubation at 37° C (Fig 3, A, B, and C). The various genera, endomyces, torula, oidia, are differentiated from the monilia group and from each other by morphologic studies and then method of reproduction (asci formation), simple budding, mycelium, and the formation of asci.

*Fermentation*—For purposes of species identification of monilia, we found the sugars indicated in Table II to give distinguishing reactions. These sugars are added to plain Liebig's extract broth in sufficient amounts to

TABLE 1\*

TYPE OF MONILIA	NUMBER AND NAME OF CULTURE	DEX TROSE	LEVU IOSE	MANN OSE	MAL TOSE	GALAC TOSE	SACCHI AROSE	I AC TOSE	INULIN	RAFFIN OSE	DEX TRIN	MYCELIUM COLONIES ON MALP AGAR IN 48 HOURS	10% GPI ATIN	MILK
I	Monilia Type I	AG	AG	AG	A	A	A	0	0	0	0	+	0	0
II	Monilia Type II	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	G
	2112 M ibicus Crank	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	801 M richmondi Shaw	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	2117 M psilosis asifordi	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	752 M pinoyl castellum	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	753 M metalindensis castellum	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
III	4021 Monilia Zilbig	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	4135 M pseudotrophic this castellum	AG	AG	AG	AG	AG	A	0	0	0	0	0	0	C
	Monilia Type III	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
	2113 M candida bonorden	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
(Lndomyces)	1369 M candida bonorden	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
	750 M tropealis castellum	AG	AG	AG	AG	AG	AG	0	0	0	0	++	0	0
	748 M metalindensis castellum	AG	AG	AG	0	AG	AG	0	AG	AG	0	+	0	0
	(Mycoderma?) 749 M krusei castellum	AGP	AGP	AGP	0	0	0	0	0	0	0	+	0	0

\*A = acid G = gas O = negative results + = positive, C = clot, and P = pedicle



make the broth a 1 per cent solution and the final  $P_H$  adjusted to 7.2. Bromothymol blue indicator is added to the broth. The broth is inoculated from a forty-eight hour agar slope culture and incubated for seven days. The cultures are observed at the end of forty-eight hours and four days but a final reading is not taken until the end of the seventh day. They are observed for the formation of acid and gas.

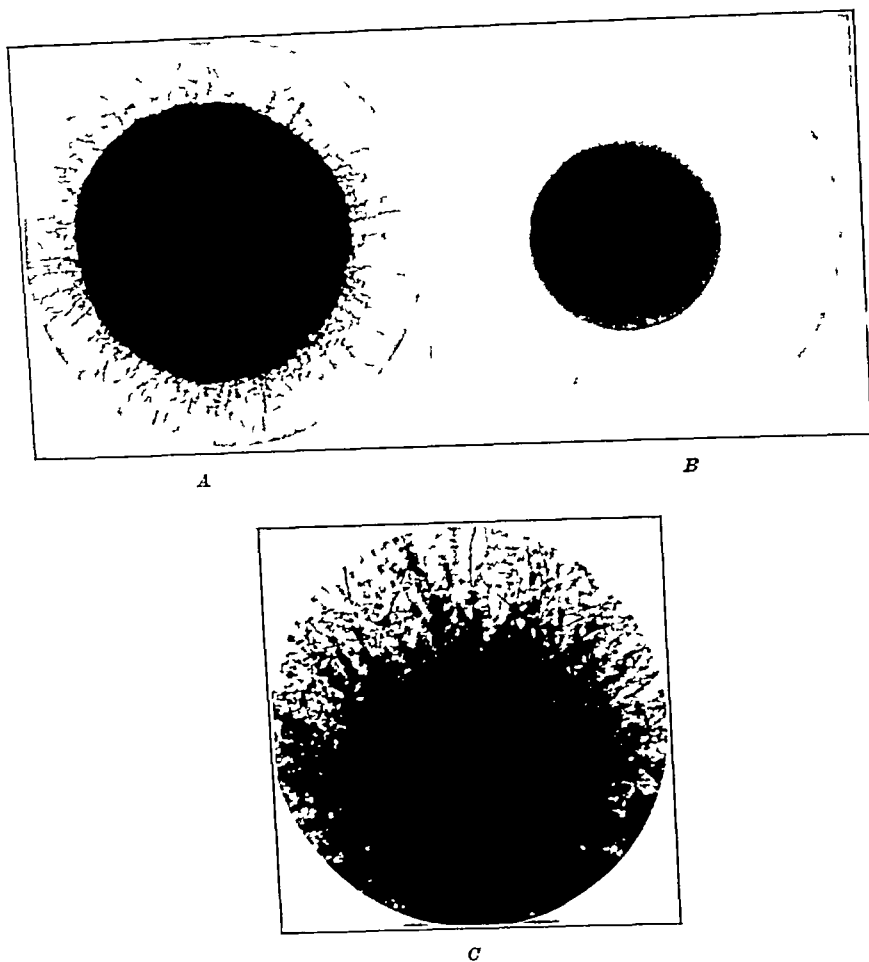


Fig. 3—A. *Monilia* Type I, forty-eight hour malt agar colony. B. *Monilia* Type II, forty-eight hour malt agar colony. C. *Monilia* Type III, colony of *M. candida*.

*Gelatin*—We have found no monilia which will liquefy 10 per cent standard gelatin and therefore we do not use it for the routine identification of species in this group.

*Milk*—We prepare a special milk media to insure constancy of results. Skimmed milk is tubed in 10 cc quantities and “Arnolded” on three successive days after which 0.5 cc of a sterile 10 per cent calcium lactate solution is added to each tube. This media is inoculated with 0.1 cc of a salt solution suspension made by washing a forty-eight hour growth from a malt agar slope, and observed for coagulation for a period of one week. The

incubation temperature is 37° C and the cultures are inspected every twenty-four hours during the test

# CLINICAL OBSERVATION

*Monilia*—The genus identification is made on the basis of morphology and method of reproduction. The species in the genus are determined by a study of the cultural behavior toward certain sugar media, calcium lactate milk, and colony morphology on malt agar after forty-eight hours incubation at 37° C.

*Morphology*—All of the cultures, 250, studied reproduce by budding and mycelial formation. The spores vary greatly in size and morphology. In the same culture small and large cells are seen as well as round and oval forms. The mycelium is septate and lateral conidia form by budding near the joints of the hyphae and terminal conidia by budding and constriction at

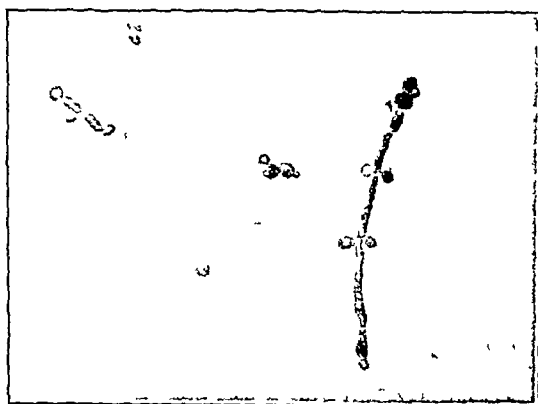


Fig 4—*Monilia* lateral and terminal conidia

the ends of the hyphae (Fig 4). We have found no morphologic variation in the genus upon which to base a species differentiation. Asepsis have not been demonstrated.

The cultural and biochemical reactions which we have found to be characteristic for species differentiation are shown in Table II.

The relation of monilia to disease has been disputed. There are, however, many clinical reports. Castellani, Ashford, Sen, Paramanand, Piper, Boggs, Pincoff, Simon, Stemfied, Johns and Shaw, and others have associated monilia as etiologic factors with bronchitis, asthma, pneumonoma, thrush, and vaginitis. In all of these reports either no attempt has been made to classify

TABLE II

SPECIES	MALTOSE	SACCHAROSE	MILK	MYCELIAL COLONIES
Type I	Acid	Acid	0	+
Type II ( <i>M. albicans</i> )	Acid and gas	Acid	Coagulation	0
Type III ( <i>M. candida</i> )	Acid and gas	Acid and gas	0	++

the fungus isolated, or Castellani's classification has been adopted. We have isolated and classified monilia from lesions in various locations in the body and have found the types described above. We have also found that in one patient vaginitis may be caused by *M. candida* and in another by *M. albicans*. This has also been true for cases of thrush, bronchitis, and asthma. While we have found Group I monilia in sputum, we have never found it to be associated with thrush or vaginitis. The brief case histories below are illustrative of our clinical experience with the monilias.

CASE 1—White male, aged fifty-two years. Came on Jan. 15, 1925, complaining of a sore mouth. He said that he had been suffering from this condition for two years and that one year previous he had been told it was due to a streptococcus infection due to bad teeth. He had his teeth removed at that time. The examination revealed a membranous exudate covering the mucous membrane of the cheeks, tongue and lips. The membrane was thin and was removed in small pieces. Direct smears stained with Gram's stain revealed a yeast-like fungus with abundant mycelium. Cultural identity, *M. candida*.

CASE 2—J. C., white male, aged five years, admitted to the hospital Feb. 4, 1931, weight 39 pounds. The parents said the child had been drooling since five months of age and had had a photophobia with yellow discharge from the eyes since one year of age. They had also noticed white patches in the mouth since he was a year old and about three years ago he had a severe infection of the mouth accompanied by acute otitis media.

The examination revealed a pale, anemic child much underweight. The corneal reflex was lost. There were a few corneal opacities in the upper half of the left cornea. In the mouth there were found many white elevated patches covering the mucous membrane and tongue. These lesions were typical of thrush. Over the palmar surfaces of the hands there was a scaling and peeling and the finger nails were thickened and discolored. These lesions were typical of epidermophyton infection. Smears made from the lesions in the mouth showed a yeast-like fungus with abundant mycelium. The cultural identity of this fungus was *M. albicans*. It was also isolated from the stool. *Epidermophyton microsporon* was isolated from the finger nails.

CASE 3—R. K., a man, aged thirty-six, a laborer, complained of coughing up blood. There was no family history of tuberculosis. Fourteen weeks before his admission he had an upper respiratory infection and had developed a cough. About a week later he had a chill following exposure. Following the chill he gradually became more short of breath and began to cough up bright red blood. He continued to cough up blood and felt weak. Three weeks later he consulted a physician who took an x-ray of his chest and told him he had tuberculosis.

Direct examination of sputum did not reveal a yeast-like fungus but the cultures grew a yeast-like fungus which was identified as *Monilia* Type I. Sputum examinations for tubercle bacilli were repeatedly negative.

CASE 4—E. S., aged fifteen, a schoolboy, admitted to the hospital Jan. 16, 1928, had developed a dry, nonproductive cough beginning about a year before. In September, 1927, he had begun to have afternoon fever which rose to about 100° F. He did not raise any sputum at this time although he still had a cough. December 12 he went to bed because of weakness. A diagnosis of bronchitis was made by the local physician. Jan. 4, 1928, the patient returned to school. He felt exhausted but there was still no sputum. January 14 he developed a high fever and for the first time raised about a teaspoonful of blood-stained sputum. The patient had lost ten pounds in the past six months. The sputum over a period of a year and a half was repeatedly positive for *M. albicans* and negative for tubercle bacilli.

CASE 5—A. W., a man, aged forty-five, was admitted to the hospital Feb. 20, 1928, in a moribund condition. His local physician said that early in December he had influenza

He was ill about four weeks at that time. He continued to cough a great deal but the sputum was not blood stained until the latter part of January. He had complained of sore throat and his voice was husky. The throat condition grew worse rapidly and his cough was almost continuous when he was admitted to the hospital. At this time he had a large ulcer involving the soft palate and another on the upper lip. Two weeks prior to his admission

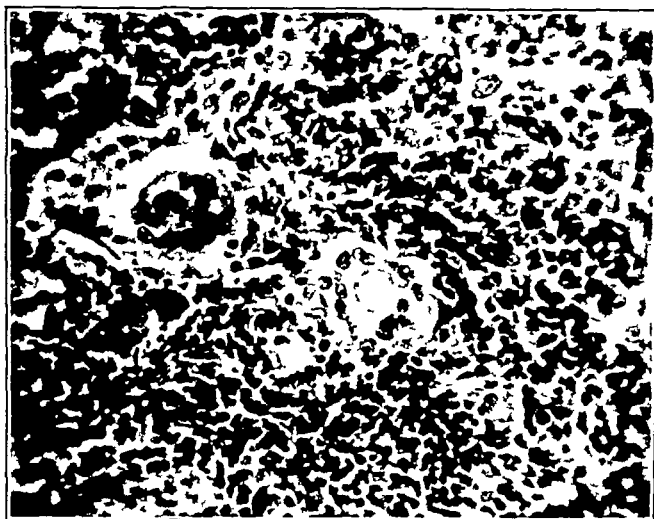


Fig 5—Blastomyces spore in a giant cell

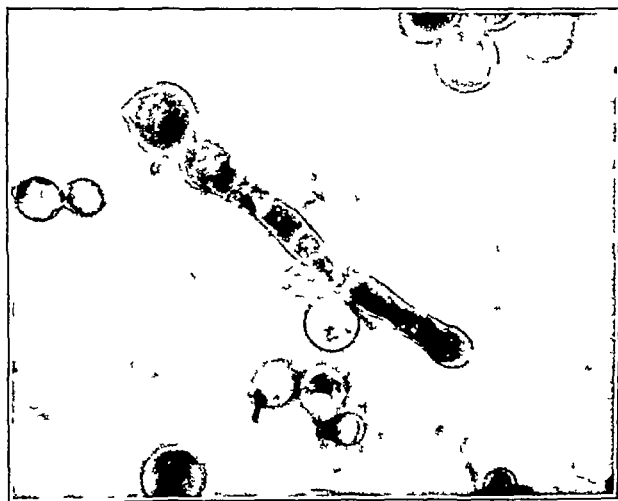


Fig 6—Blastomyces (Oidia) septation and abstraction of hyphae

to the hospital a direct examination of the sputum revealed large numbers of yeast like cells and mycelial threads in the sputum. The cultural results identified *M. albicans*. Examination of secretion from the ulcers revealed the same organisms.

*Oidium*—(Blastomyces) We have isolated two cultures and with these have studied one, *Asteroides*, Castellani, secured from the American type cul-

ture collection. The fermentation reaction, colony morphology and other cultural characteristics are described in Table III. This organism we believe to be the same as the one described by Busse and Gilchrist as the cause of blastomycosis. In tissue this organism is seen as single, large spores, 8 to 10 microns in diameter, some of which can be seen to be budding. They are often seen in the giant cells which are part of the tissue reaction (Fig. 5). Cultured on solid media it soon produces profuse mycelium, the colony finally being covered by a downy, fuzzy growth. Spores are formed from the mycelium by abstriction and septation of the hyphae (Fig. 6). This organism is easily distinguished from the other yeast-like fungi described in this paper by the size of the spores and their double contoured wall and by the very coarse mycelium. The fermentation further differentiates this organism. It ferments no sugar even after long periods of incubation thirty days.

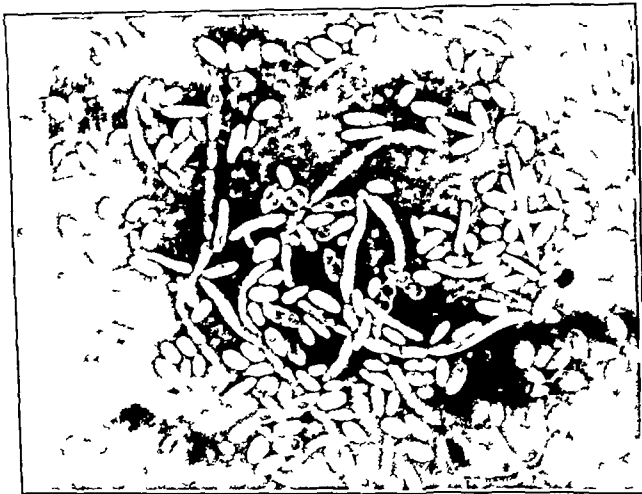


FIG. 7.—Asci in culture of *Endomyces*.

CASE 1—C. G., aged seventeen years, admitted to the hospital on Sept. 3, 1929, complaining of pain in the chest. He had been ill since May, 1928. He had been in bed since September, 1928. He had impairment of function of joints of the hands, knees, elbows and shoulders since August, 1929. The physical examination revealed a poorly nourished and anemic male, marked lag in respiration in the left chest, impaired resonance in both apices, more marked on the left, tactile and vocal fremitus decreased over the same area, high pitched inspiration and prolonged expiration, systolic murmur over the aortic area transmitted to the vessels of the neck, liver palpable one and one half centimeters below the costal margin, edema of the lower extremities, fluid in both knee joints, hypertrophic joint changes in the knees, feet, and hands. Probable diagnosis at this point, pulmonary tuberculosis, chronic infectious arthritis, secondary anemia, chronic pharyngitis, and dental sepsis.

The sputum examination was negative for tubercle bacilli after many specimens had been examined. The direct examination of the sputum showed large double contoured spores and no mycelium. The cultural identity of the organism was *oidium* (blastomycetes). Clinical diagnosis, blastomycosis.

*Endomyces*.—This organism reproduces by budding and mycelial and asciform formation. The mycelium is septate and conidia are formed laterally and at

the joints of the hyphae, and by abstraction at the ends of the hyphae. There are minor morphologic differences between this organism and the monilia. The chief distinguishing characteristics are the production of asci (Fig. 7) and the sugar fermentation.

It has been our experience that a yeast-like fungus giving the fermentations recorded for this organism in Table III has always shown asci and has turned out to be endomyces. We have now isolated this organism from three specimens of sputum. The study of three cultures is, of course, a limited experience. However, the fermentation of lactose, milk, and raffinose

TABLE III

	GLUCOSE	SUCROSE	MANNITOL	MILK	LACTOSE	SUCROSE	LACTOSE	INULIN	RAFFINOSE	DEFECIN	GPI MIN	MILA	MYCETAL COLONIES MAY 18 HOURS
Monilia Type I	AG	AG	AG	1	1	A	0	0	0	0	0	0	+
Monilia Type II	AG	AG	AG	AG	AG	A	0	0	0	0	0	C	0
Monilia Type III	AG	AG	AG	AG	AG	AG	0	0	0	0	0	0	++
Endomyces	AG	AG	AG	0	AG	AG	AG	AG	AG	0	+	A	+
Saccharomyces	AG	AG	AG	AG	AG	AG	0	0	AG	0	0	0	0
Oidia	0	0	0	0	0	0	0	0	0	0	+	0	+
Torula	A	1	A	0	A	A	0	0	0	0	0	0	12 days 0

has been a constant characteristic of these three strains. The organism secured from the American type culture collection, classified as *M. macedoniensis*, produced asci in our study and we therefore recognized it as an endomycete. It did not, however, ferment lactose. It appears then that a study of a large number of strains might reveal a variety of species.

CASE A S—This patient, a woman thirty-five years of age, was admitted to the hospital on Dec. 11, 1927, with a severe rhinopharyngitis and bronchitis. After two or three days in bed she appeared to be improved but she continued to have a low fever and a few rales at the base of both lungs. This condition persisted for two more days when an area of consolidation was found in the right lung posteriorly. The patient's fever continued about the same, 99.6°-101°, but the chest manifestations did not improve.

X-ray plates made December 29 showed enlargement of hilum lymph nodes on the right side, increased visualization of peribronchial structures, several organized spots scattered through the lower half of the right lung, and a fogging in the left apex.

Sputum was sent to the laboratory December 23 with a request for an examination for acid fast bacilli. This was repeated for five successive days and each time reported negative. However, on December 26 the laboratory reported the presence of a yeast-like fungus. The organism proved to be an endomycete.

The patient recovered and was discharged from the hospital on December 20. She has been followed closely and at the present time x-ray plates of her chest are clear and she is in good health.

*Torula*—We have studied six cultures, four isolated by us and two from the American type culture collection. The morphology of this organism is like that of *saccharomyces* except that no asci have been demonstrated. It reproduces chiefly by budding. However, in broth cultures left at room temperature for three months abundant mycelium is produced in the sugars in which acid is produced. No gas is formed in sugars, acid being produced slowly in glucose, levulose, mannose, galactose, and saccharose. We have encountered two varieties of colonies, one a creamy, glossy colony, and the other a salmon to pink color. The colonies are more glossy than the monilias and *saccharomyces*.

CASE J. L.—This was a white male patient, twenty-three years of age. He has suffered from asthma ever since he was two and one-half years old. His asthma followed an attack of pneumonia. His condition had not increased in severity. He sometimes had quite severe attacks of dyspnea. He said he had noticed a sensitiveness to various dusts and to the emanations from cats and dogs.

The physical examination revealed a congested nasal and pharyngeal mucosa and emphysematous lungs.

The laboratory examinations were essentially negative, except for the sputum examination. The x-ray showed nothing important. The sputum cultures revealed a yeast-like fungus, which was identified as *torula* (white).

#### SUMMARY

This paper briefly reviews the literature on the classification of yeast-like fungi and points out the confusion which has led to a multiplicity of species for monilias and resulted in the classification of all yeast-like fungi as blastomyces.

The methods used and the characteristic differences of certain of these types of fungi are described. The differences are found to be characteristic for the various species and when the organisms are observed under the conditions described, they are constant in their behavior, showing no variation after artificial cultivation over a period of many years.

This study is associated with a brief report of cases infected with different kinds of yeast-like fungi. These cases indicate the clinical manifestations of these infections. This report suggests that these organisms may be the cause of bronchial asthma in certain cases. The cases also demonstrate that these organisms may be associated with lesions in various anatomic locations, that is *M. candida* may in one patient be associated with thrush and in another with vaginitis.

This study further indicates that yeast-like fungi can by careful clinical and laboratory studies be established as the primary etiologic agent in certain cases of pulmonary, vaginal and mouth mucous membrane inflammatory processes. On the other hand such cases as J. L. (*torula*) raise the question as to the significance of this organism in an allergic patient.

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## ON THE ADVANTAGE OF ALTERNATING THE VEGETABLE AND METALLIC DIURETICS IN THE TREATMENT OF EDEMA OF CONGESTIVE HEART FAILURE\*

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**I**N THE prosecution of our studies<sup>1</sup> on the mechanism of the action of various types of diuretics we were impressed with the influence that the order or sequence in which the drugs were administered had upon the type and degree of diuresis. In our second (repeated) compound or triple drug experiment (William Blake) our results as to the filtration and reabsorption seemed to refute our original findings and conceptions, but the idea of a possibility of "hang over" or persistence of effects was immediately conceived and steps were taken to verify such an explanation or contention (Tables I II, and III, Figs 1, 2 3 and 4)

This evidence we felt would in turn help to substantiate the conception of the purine's increased glomerular filtration and the heavy metal's decreased tubular reabsorption mechanisms of diuresis with which we were concerned. These observations and facts suggested a possible complementary therapeutic combination of the two modes of action by a proper sequential or simultaneous administration of the two types of diuretics.

It was evident that augmentation of diuresis under such would have a distinctly practical value and should be put to extensive clinical investigation.

This has been done in the medical wards of the John Sealy Hospital and the validity of the arguments seems to us to be established almost beyond peradventure. We can administer combinations which almost uniformly

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yielded better results than did the two drugs previously administered alone. We have also been successful in instances where one drug alone in the same dosage had failed. In general it furthermore has been possible to accomplish diuresis with smaller doses of the drugs doses that for either drug alone would be ineffectual. The great enhancement of the urinary output brought about as a result of purposeful premeditated arrangement of the time and order of drug administration by virtue of fact that the drugs in our way of thinking act on different parts of the renal unit struck us as a distinctly practical therapeutic procedure.

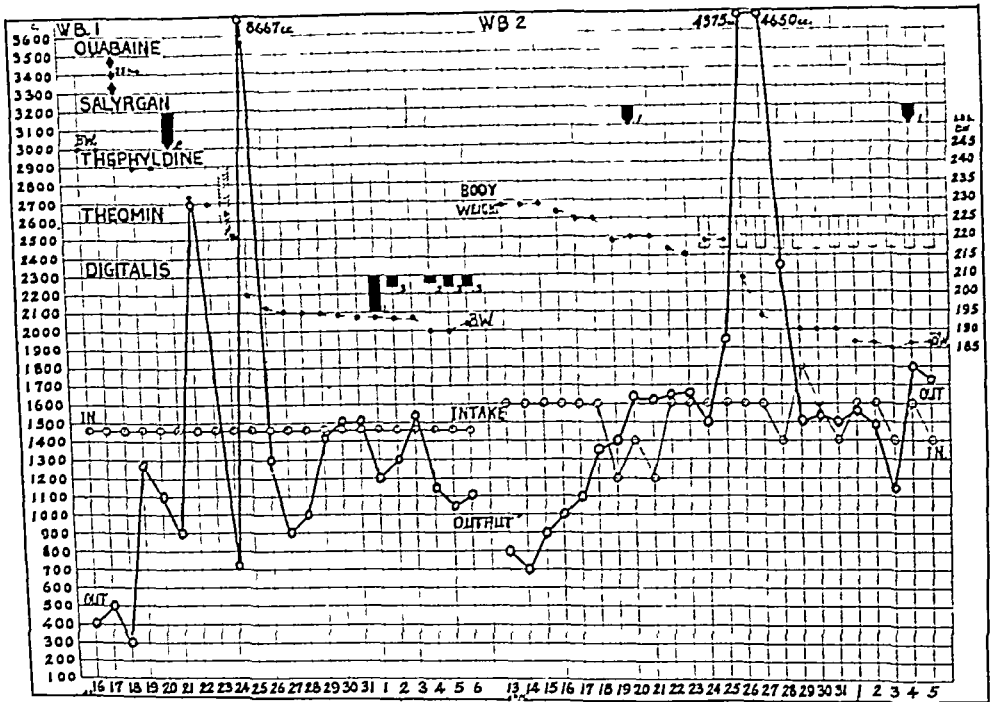


Fig. 1—Clinical observation of the daily body weight fluid intake and urine output over a period of twenty-two days of the first and twenty-four days of the second admission of W. B. colored aged forty-three with syphilitic aortic regurgitation in extreme congestive failure with anasarca. Complete studies were made by the Rehberg creatinine method of the mechanism of the diuresis following the intravenous injection first of ouabaine second three days later of salyrgan and three days after this the third acute experiment was done with theophylline ethylene diamine and finally digitalization was carried out. The results of the acute experiments with regard to (F) filtration and (R) reabsorption will be shown in Figs. 2 and 4. Note the great augmentation of the output that resulted from the theophylline that followed the mercurial. The second admission shows the efficiency of theophylline by mouth following the mercurial by vein.

Just as Schmitz<sup>2</sup> using the Rehberg<sup>3</sup> creatinine method in the study of the mechanism of experimental diuresis in animals has shown, so we too have demonstrated in patients with congestive heart failure and edema that the metallic diuretics, especially the mercurials, accomplish their results by adversely affecting the permeability of the tubular epithelium and decreasing the tubular reabsorption primarily, while the purine derivatives, especially theophylline and in our experience even digitalis bodies, produce diuresis principally by increasing the glomerular filtration. The opening up of more renal

units by the vascular effects of the xanthenes probably also plays a minor part as does the increased rate of flow through the tubular capillary plexus in the increased efficiency of urine excretion.

Theophylline ethylene diamine (theophylline), theophylline, the drug in the purine or xanthine series that we have used most often has definite augmentory central circulatory, as well as renal effects. It is primarily used as a vasodilator and is generally considered to have its effects on the active arteriolar vascular sector in the heart and kidney, but probably also in the other peripheral parts of the vascular beds. Mercury salicyl-allylamide o-acetate of sodium meisalyl (salvigan), the mercurial used is apparently fil-

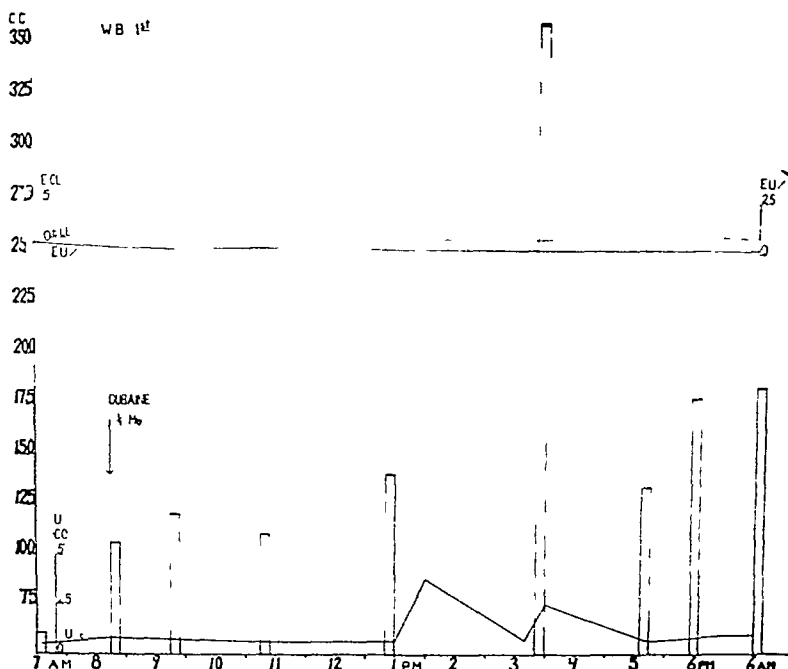


Fig. 2.—A schematic representation of the results obtained by the creatinine method of estimation glomerular filtration ( $F$ ) and tubular reabsorption ( $R$ ) following the intravenous injection of ouabaine. The total heights of the columns to the very top represents the filtration rate ( $F$ ) per minute in cubic centimeters; the columns to the lower margins of the black cap represent the reabsorption ( $R$ ) rate per minute in cubic centimeters; the black cap itself the difference between  $F$  and  $R$  is the urinary output rate in cubic centimeters per minute ( $U$ ). The actual urinary excretion per minute on a scale 20 times that used for  $F$ ,  $R$  and  $U$  is shown by the connected line at the bottom.  $U$  cc obtained by dividing each urinary output in cubic centimeters by its interval in minutes and charting the same opposite the time at which each specimen was voided.

In the upper part of the graph there is charted the percentage of the filtered urea (EU per cent) and chlorides (ECI per cent) excreted at the time intervals throughout the day.

Note that after ouabaine just as was the case with digitalin there was a striking increase in filtration and only a slight decrease in reabsorption slightly delayed possibly because it depended on central or cardiac improvement in circulation and later improvement in glomerular circulation.

tered in dilute solution through the glomerular membrane, and as it comes in contact with the tubular epithelium and is diffused through in minute amount or reabsorbed, it affects the protoplasm of the cells of the tubular membrane and decreases their permeability. If the dose of mercury is too great, or the cells of the tubular epithelium are unusually susceptible, destruction results,

and there is an exaggerated or complete permeability with a resulting anuria. This has apparently happened in a few of our cases in which large 2 cc doses of salyrgan were given intravenously. Upon the basis of these facts of the *modus operandi* of each type of diuretic the results of our experiment served to substantiate our contentions. We have studied these combination therapeutic effects in a series of 13 successfully and 2 unsuccessfully treated patients in congestive heart failure.

#### METHOD

All edematous patients admitted to the medical wards of the John Seely Hospital are strictly confined to bed with a back rest and pillows as desired to make them comfortable. A low protein (40G) salt poor diet (2G) is prescribed with 200 cc of fluid at two or three

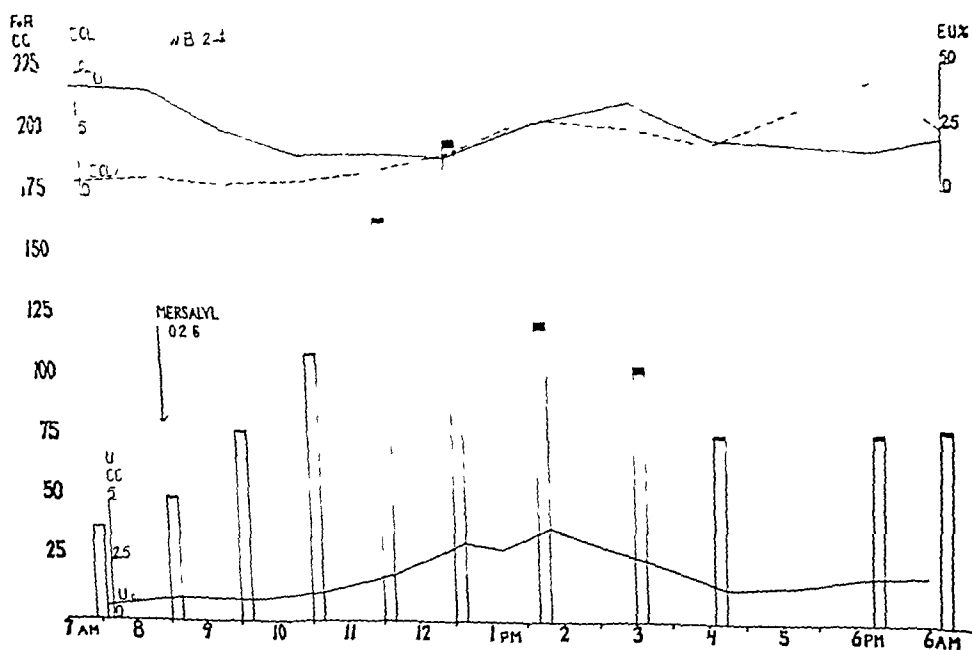


Fig 3—The description given for the symbols in Fig 2 will suffice for this graph. In the experiment depicted here the second one the mercurial salyrgan was injected intravenously and the diuresis was accomplished not in the pure salyrgan way by depressing tubular absorption (increase in black caps) but there was an increase in filtration which was considered as a persistence of the general circulation improvement effect of the ouabaine previously given.

hourly intervals for 8 or 5 administrations according to the degree of edema that the patient presents totaling 1600 cc or 1000 cc for the twenty four hour period. The degree of edema is roughly estimated as Grade 1 when there is just pitting, Grade 2 when it extends to the knees, Grade 3, to the scrotum, Grade 4, to the back with ascites, Grade 5, general anasarca. The body weight and height are recorded. All urine specimens are collected separately ending always a twelve hour period by emptying the bladder at 6 PM and 6 AM to complete the twelve and twenty four hour outputs. Morphine sulphate is used ad lib to quiet and keep the patient at ease.

On the first morning in the hospital before breakfast, a 30 cc blood sample is taken without stasis and under mineral oil for complete chemical analysis, two urine samples at

known time intervals are collected for volume rate per minute and urea clearance determination, and the complete twelve and twenty four hour specimens are chemically analyzed.

The patient then is kept at rest in bed for three days on the same diet with the intake of fluid output of urine and the body weight recorded and charted daily.

If after three days, there is no rise in the output, the regime of experimental therapeutics is begun. If, however, there is any indication of a rising level of urinary output, the experiment is postponed for as many days as seems necessary to establish a baseline. On the first experimental day, the 30 cc of blood and the first two urine specimens and the twelve and the twenty four hour specimens are again collected for analysis, and then the drug is given.

Theophylline ethylene diamine 0.45 gm in 10 cc of physiologic salt solution is introduced intravenously and the time recorded. Each urine specimen voided, thereafter, is col-

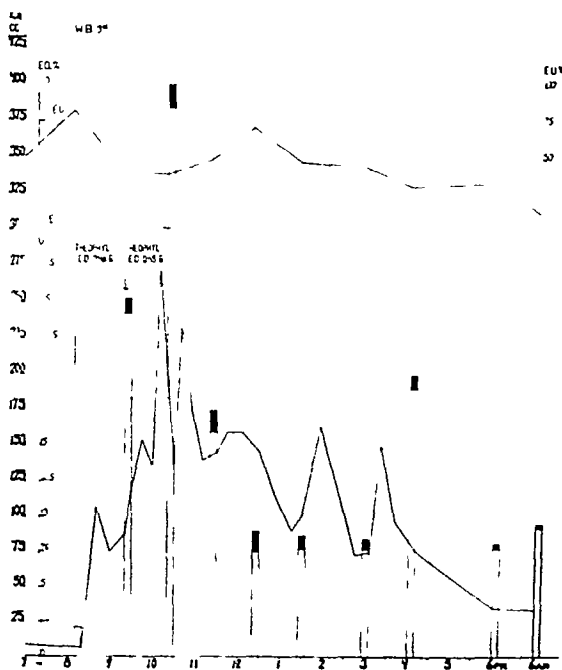


Fig 4—Again the description of the graph given under Fig 2 is to be applied here. The third experiment the result of injecting theophylline shows a tremendous increase in the glomerular filtration rate to a maximum of 400 cc with this the strikingly prominent black caps indicate a conspicuous defect in reabsorption, a persistence of the previous mercurial effect with as a result a diuresis of almost nine liters of urine in twenty-four hours.

lected in a separate or individual bottle, the time is noted, the interval calculated in minutes, the urine volume and specific gravity are determined, and the rate per minute estimated. From this data can be determined, the time after injection of onset of diuresis or augmented or increased urinary flow, the time interval (after injection) elapsing before the maximum diuresis is obtained and the length of the diuretic period on this control dose of theophylline ethylene diamine (0.45 gm) or of any other drug that may be chosen. The second experimental period is a similar one but for the change in the drug introduced intravenously.

As soon as the baseline of urinary excretion is reached and the glomerular filtration effect has disappeared, which is usually within a day except for digitalization effects that are more persistent, the second drug, the mercurial, is administered under the conditions of

the first experimental day. The preinjection blood and urine samples are taken for analysis, 1 cc of a 10 per cent solution (0.1 gm) of salyrgan is injected, all urine voidings are collected separately again, and the time of onset of maximum flow and the length of time of the diuretic action of the drug are noted.

The third experimental period consisting of the second or postsalyrgan injection of theophylline, which is to show the combination effect of salyrgan followed by theophylline, is inaugurated. The control blood and urine samples are collected again. The same dose of theophylline previously used is injected intravenously and the time of onset of maximum flow and the length of the diuretic period are recorded. By comparison of these figures the cumulative effects are to be noted albeit roughly, but always definitely.

In some patients the salyrgan was given only a few hours before the theophylline and occasionally the drugs were used simultaneously.

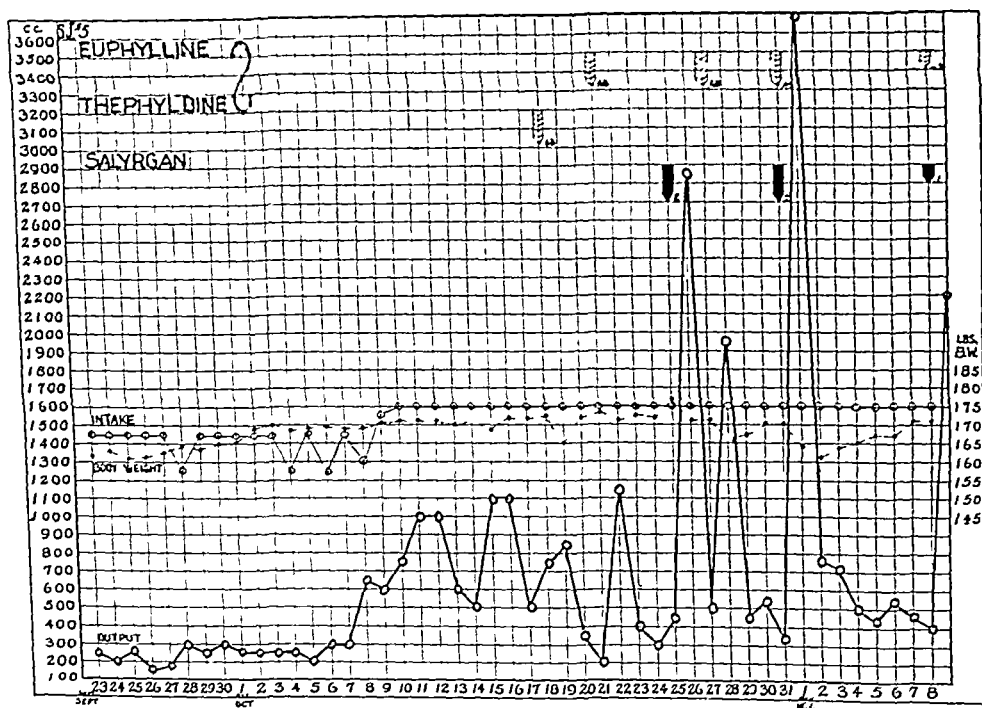


Fig 5—The daily record of a patient (S. J.) with hypertension, coronary arteriosclerotic heart disease with congestive failure, cardiac cirrhosis of the liver and ascites. After a long rest period two theophylline injections of 0.48 gm each were given at three day intervals with but slight effect upon the urinary output. Salyrgan 0.2 gm was then injected and a fair diuresis resulted. On the following day theophylline produced twice as much of an output than it had previously. Four days later the drugs were given during the same morning a few hours apart and a good diuresis resulted. Eight days later the drugs in half the dosage previously given had about two-thirds of the effect. The first was the only dose of euphyllin (the German product) the others were all theophylline (the American preparation).

A fourth and fifth series of experiments were carried out with the hope of obviating the necessity of the intravenous injection of the drugs.

In the fourth series the patients were given a control injection of 0.1 gm salyrgan or were put directly on one of purine derivatives, that is used for oral administration and the effects were noted and recorded. This drug is administered continuously three or four times a day according to the preparation used in doses of 0.1 to 0.5 gm ( $1\frac{1}{2}$  to  $7\frac{1}{2}$  gr) until the output of urine was about at a constant baseline for three or four days. Then an injection of 0.1 gm (1 cc) salyrgan was given and the rise in urinary output recorded. The

purine was continued through the mercurial day and for several days thereafter, and the results were charted

In the fifth series the purine preparation was continued for a control period of several days and a mercury and chalk pill 0.0065 gm (1 gr) was given along with the purine by mouth three times daily for four to seven days and then the mercury administration was discontinued and the purine alone was continued for several more days

### RESULTS

Tables IV and V are so constructed as to represent a continuous story of the study of a patient. Each hospital day, the therapy and resulting fluid exchange is accounted for, reading from left to right and from the end of

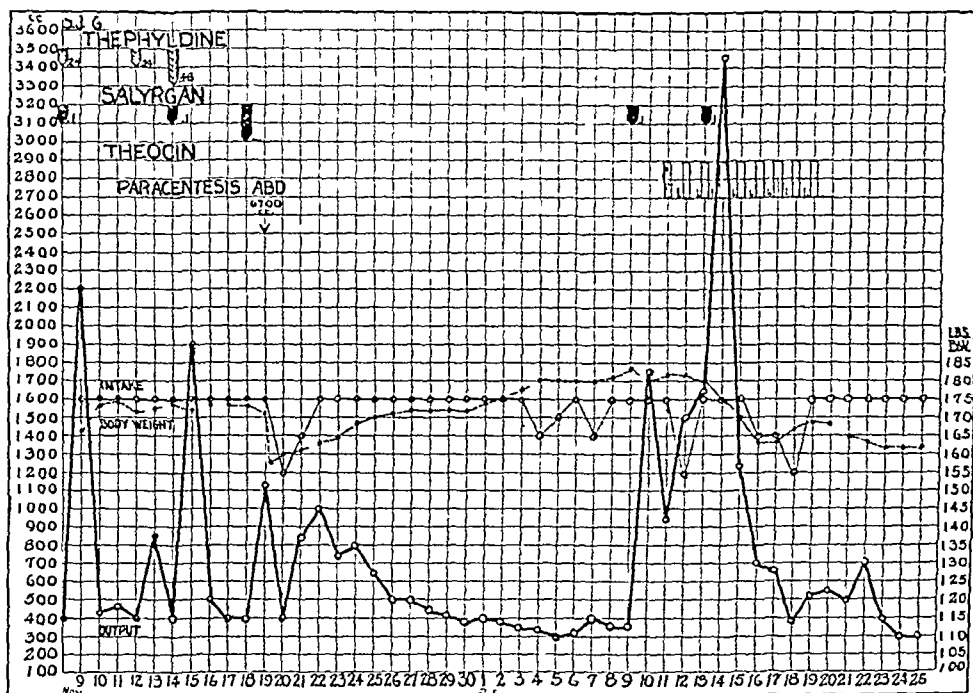


Fig 6—A continuation of the above record showing the result of the last combination of half doses of salyrgan and theophylline. A second combination with the full dose of theophylline and the half dose of salyrgan was less effective than the previous half and half experiment and following this the full 0.2 gm dose of salyrgan resulted in but half of the urine output. Abdominal paracentesis was done and 6700 cc removed. Subsequently 0.1 gm salyrgan produced some diuresis but the same dose following three days of theophylline by mouth was doubly effective.

one line to the beginning of the next. The weight is frequently given as a rough index of the degree of or changes in edema and serves as a check on the results. It must be remembered that each succeeding diuretic experiment, especially when one succeeds another within a few days, begins under less favorable auspices for the edema in the tissue interstices has generally been reduced. The number of days of rest at the beginning and after each therapeutic attempt and the average urinary outputs per twenty-four hours for these days are recorded and taken as the baseline for calculations of the percentage of increase as the result of the drug administration.



TABLE II

TIME	IN P	VOI	N <sub>2</sub> ClN (Cl)		CO <sub>2</sub>	U U %		U S %		CR		G	F	R	F U	U U		R U	UR %	E U %	C U	F C I	U C I	R C I	GLR %	E C I %	O C I
			U %	N %		U %	S %	U %	S %	U %	U %																
U 6 00 7 35	05	62	0 65	120	561	54	570	21 4	112 0	1 87	50 8	30 1	38 4	8 35	3 7	1 7	12 2	44 3	26 6	210	2 70	217 2	560	1 27	0 704		
U 7 25																											
U 8 10	65	61	0 90	420	528	52	522	23 1	226 0	1 98	52 8	52 0	51 0	12 0	5 11	6 0	13 5	42 8	22 6	275	1 23	270 8	531	1 54	0 812		
U 8 30																											
U 9 40	00	52	0 87	120	512	47	600	25 0	280 0	1 16	91 5	70 3	74 4	10 8	5 20	11 6	15 6	20 3	21 0	100	3 72	402 3	714	0 092	0 888		
U 9 30																											
U 10 40	00	08	1 13	528	512	10	102	30 0	270 0	2 56	97 6	110 4	100 3	13 1	5 22	27 0	25 5	15 8	15 4	605	5 07	550 0	512	1 06	1 03		
U 10 30																											
U 11 10	60	111	1 00	702	514	12	300	22 2	193 0	2 22	57 0	165 2	103 3	16 7	5 70	31 0	19 0	15 5	13 5	900	15 1	951 0	601	1 65	1 46		
U 11 30																											
U 12 40	60	105	1 25	501	405	18	200	23 1	134 0	2 22	60 4	180 2	103 0	15 3	6 50	38 8	20 1	11 3	5 66	972	20 0	943 0	488	2 05	1 80		
U 1 50																											
U 3 10	70	213	3 17	976	462	52	178	16 5	92 5	2 61	75 4	122 0	110 4	23 1	6 15	10 9	11 2	20 8	9 40	568	32 2	535 5	115	5 70	2 08		
U 3 00	80	221	2 76	921	495	46	208	15 8	103 0	2 72	37 0	101 0	101 8	16 5	5 74	10 8	10 6	11 2	518	25 5	102 5	153	1 02	1 87			
U 4 15																											
U 4 05	05	00	1 38	888	440	45	800	27 3	125 5	2 31	75 6	77 0	75 6	21 0	1 11	16 5	22 2	10 7	11 0	117	12 3	311 7	413	1 55	1 80		
U 5 15																											
U 6 15	120	204	1 70	1 86	303	46	154	27 3	105 0	2 31	15 5	77 1	75 7	21 1	3 12	18 0	21 8	11 4	6 71	981	23 6	257 1	110	8 1	3 82		
{ 6 00 1 M 6 00 A M 8 00 A M																											
	720	1300	1 93	924	102	52	218	20 0	80 0	1 01	41 2	70 1	77 5	21 7	1 15	17 5	22 6	19 4	8 0	307	17 8	219 2	22	4 85	2 0		

TABLE III

TIME		IN	VOI	U	N (C) N (C)	CO <sub>2</sub>	U U %	U S %	CR U %	CR S %	G	F	R	F U	U U	R U	UR %	F U %	C U	F C I	U C I	R C I	F C I %	G C I		
U	6 00 7 00	60	10	0 52	363	577	53	333 0	15 0	115 0	2 61	11 1	36 0	35 2	5 10	2 71	2 7	7 68	50 0	22 2	20 3	2 00	202	573	1 11	0 63
B1	6 50	50	53	0 06	603	511	48	487 0	11 0	103 0	6 0	32 2	21 1	20 7	3 12	2 50	0 50	2 78	82 0	20 5	1105	1 02	1123	512	0 307	1 27
U	8 10	70	757	10 8	428	528	18	112 0	11 3	74 0	3 10	23 1	253 0	142 2	10 2	11 3	21 0	9 5	39 6	9 23	1338	1 63	1 33	570	0 117	0 81
B1	9 20	00	950	16 31	500	512	40	113 0	12 0	73 5	3 0	14 5	100 0	84 7	18 0	18 5	20 5	7 7	38 0	9 10	2018	61 7	1083	517	0 110	0 773
U	10 30	00	1002	17 0	162	511	51	600 0	12 3	30 5	3 75	10 5	175 3	150 6	21 3	10 2	11 1	6 05	48 0	1 88	900	78 5	882	573	0 52	0 85
B1	11 30	00	920	15 32	462	577	50	500 0	12 0	23 5	1 0	5 88	40 2	75 0	10 8	7 00	3 1	1 0	71 0	1 17	520	70 5	150	600	1 555	0 80
U	1 25	00	611	10 22	194	577	10	10 2	11 5	25 5	3 0	8 5	87 0	76 8	10 0	1 72	5 5	7 17	17 2	4 00	502	50 6	151	500	1 01	0 850
B1	2 55	05	780	8 22	658	627		600 0	13 0	29 5	2 56	10 32	81 8	70 6	11 13	1 03	7 1	9 2	14 0	1 62	532	51 2	478	622	1 02	1 05
U	4 10	05	650	10 0	627	363		75 0	12 0	10 0	1 50	0 0	203 0	190 0	21 0	7 50	10 5	8 7	31 2	6 2	720	62 7	603	138	0 77	1 73
B1	6 00	110	162	1 20	627	394		75 0	11 5	31 8	1 06	10 3	81 2	77 0	9 35	1 15	6 2	8 0	9 17	6 52	102	20 1	377	188	0 66	1 27
U	6 00 1 M																									
B1	6 00 A M																									
U	8 00 A M	720	2100	1 31	658	491		26 1	11 2	31 5	1 57	28 16	61 7	91 3	10 6	1 28	9 3	10 8	12 1	2 31	107	22 3	145	190	0 178	1 33



If a spontaneous diuresis occurs or if the effects of a diuretic drug persist, the baseline will be high and the percentage of increase less striking. The percentage increase must therefore be considered with what has gone on previously and the actual urinary volumes should also be studied, and compared with the initial twenty-four hour excretion amounts with full cognizance of the effects of previous diuresis. Therefore a direct comparison of percentage increases cannot be made. (See Figs 5, 6, and 7)

TABLE IV  
THEOPHYLLINE IV AND MERCURY IV

	CONTROL REST PRELIMINARY			THEOPHYLLINE IV				REST POST-D WITH T			MERCURY IV			
	BODY WT	DAYS	AVERAGE OUTPUT	DRUG	DOSE gm	RF SUIT cc	PER CENT INCREASE	BODY WT lb	DAYS	AVERAGE OUTPUT cc	DRUG	DOSE gm	RE SUIT cc	PER CENT INCREASE
S J	173	2	250	EUP*	0.48	1060	325	172	3	330	S	0.2	2833	760
	171	1	500	TPD	0.48	1814	263	170	3	425	TPD	0.48	3994	840
								171	3	456	TPD	0.24	2230	389
	174	3	433	TPD	0.24	915	112	174	1	400	TPD	0.48	1890	373
F J	132	3	320	TPD	0.48	1780	394	132	1	1100	S	0.2	4660	324
	132	2	525	TPD	0.48	4540	765	130	2	620	S	0.1	2431	292
	126	1	250	TPD	0.48	1150	360	125						
								166	3	742	S	0.1	2522	241
W S	156	1	860	TPD	0.48	2682	213	150						
L S	146	7	320	TPD	0.48	953	197	146						
	146	2	1380	TPD	0.48	1696	23	145	1	1377	S	0.1	2737	102
	140	1	1050	TPD	0.48	4086	290	137	1	3173				
M H	149	S D												
	144	3	2020	TPD	0.48	2850	41	141	1	1960	S	0.2	3200	65
	141	2	1467	TPD	0.48	2212	51	140	2	1766	S	0.1	3316	89
	140	1	1955	TPD	0.48	1911	0	140	3	1280				
C B	155	4	395	TPD	0.48	875	122	153	1	1100	S	0.1	1800	64
	150	1	500	TPD	0.48	2500	400	144	9	650	S	0.1	2500	285
								137	13	714	S	0.1	3200	350
S Q	149	8	516	AMP	0.48	2104	314	140	1	1200	S	0.1	1203	0
	139	0	1203	AMP	0.48	2641	108	130	2	1120	S	0.1	1438	100

\*Drugs

EUP—Euphyllin Theophylline ethylene diamine

TPD—Theophylline Theophylline ethylene diamine

AMP—Aminophyllin Theophylline ethylene diamine

IV—Intravenously

Post-D with T—After diuresis with a theophylline preparation

T—Theophyllin

S—Salyrgan

S D—Spontaneous diuresis

A glance at Table IV will reveal increases of from 100 per cent to 400 per cent with ranges mostly between 250 per cent and 350 per cent regardless of rises in the baseline output and decreased dosages. The greatest increase of 840 per cent was obtained in the fourth experiment in Case S J when the maximum doses of theophylline and salyrgan were given intravenously on the same day and the former preceding the latter by only a few hours.

In the fifth experiment three days later with the minimal doses for each

drug exactly half of those used in the fourth experiment a yield of 389 per cent increase was obtained Three days later the half dose of theophylline alone intravenously gave a 112 per cent increased response and the next day the combination of the full dose 0.48 gm of theophylline and 0.1 gm salyrgan yielded a 373 per cent rise in output and three days after this 0.2 gm salyrgan alone intravenously produced 192 per cent more than usual, and finally after twenty days' rest during which the body weight increased 9 pounds, an injection of 0.1 gm of salyrgan enhanced the output 244 per cent

The second case of F. J. shows the results just as strikingly as do also V. S., L. S. and C. B. Case 6, M. H., had a spontaneous diuresis at the very

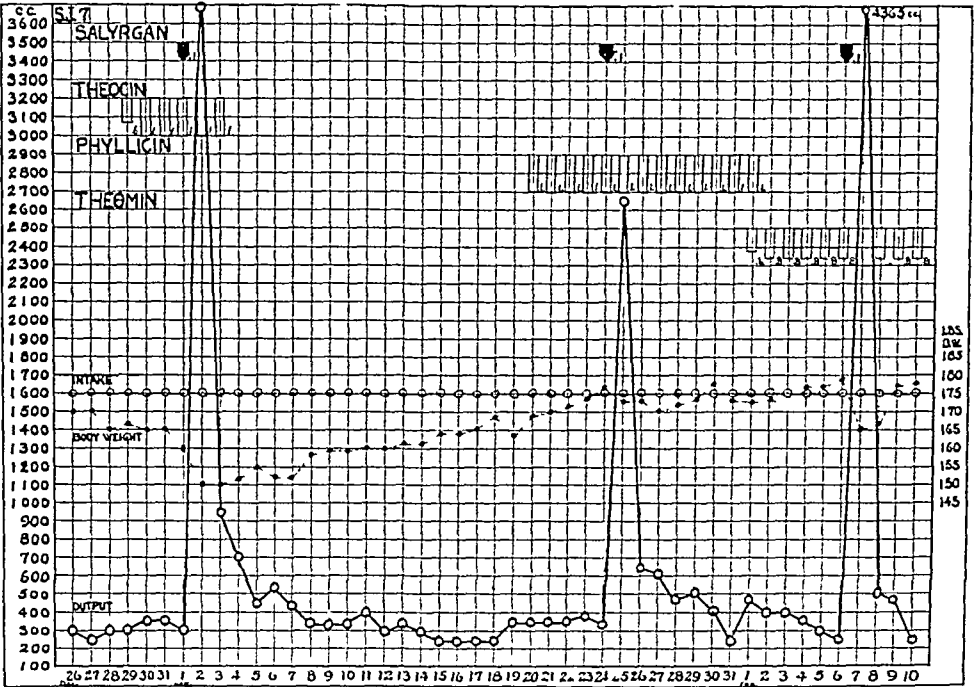


Fig 7—A continuation of the above record presenting a comparison of the diuretic effects of the combinations of theophylline in preparations by mouth before during and after injections intravenously of 0.1 gm (1/2 gm or 1 cc) of salyrgan

beginning which removed more than half of her total weight loss and therefore made the effects of the drugs much less striking Case 7, S. Q., was treated with another preparation and at a lower dosage Case 8, V. W., and 9, F. L. M., failed to respond to any of the diuretics and went to exitus with anuria following salyrgan

Table V shows the results obtained in four patients, most of whom were repeatedly studied, during the use of a purine derivative alone by mouth over a period of three to six days then a mercurial usually salyrgan is added by injection on one day the results noted and the purine continued alone for several days The results of the immediately preceding experiment are given first because most of these patients had been diuresed before the

TABLE V  
PURINE P O AND MERCURY IV

TRICLIDING EXPERIMENT				PURINE P O ONLY				MERCURY IV + PURINE P O				PURINE ONLY DRUG				
RW	IRRAI	CO	AVERAGE	BODY	DRUG	DAYS	AVF	BODY	DRUG	DOSE	REL	IN	BODY	DOSE	DAYS	AVF
IN	DRUG	OUT	OUT	WT	WT	WT	OUT	WT	WT	WT	WT	WT	WT	WT	WT	WT
S J	173 S O 2	1120	20 0 495	180	TC*	10	1333	182	S	gm 0.1	603	171%	175	TC	10	730
	157 Purine	6700	1 S 0 1g 1712	170	TC	10	330	165	S	0.1	1250	1190%	150	TC	10	940
			9 0 105	170	PC	10	360	173	S	0.1	2560	612%	170	PC	10	560
			15 0 302	173	TM	0.5	370	177	S	0.1	1300	1060%	166	TM	0.9	110
S Q				183	TBS	10	258	186	S	0.1	2550	590%	181	TBS	10	600
				193	TBC	15	507	192	S	0.1	2735	138%	191		15	1000
	115 Amp	0.45	1 0 550	140	TC	10	2066	122	S	0.1	2700	15%	117	TC	0.1	1100
C B	153 TPD	0.15	5 0 1055	140	TC	10	802	150	S	0.1	2500	190%	113	TC	10	915
		7500			TC	10	935	146	HgCl <sub>2</sub>	0.0065	950	2%	115	TC	10	680
					TC	10	680	141	HgCN	0.0100	2500	265%	147	TC	10	650
					TC	10	650	112	S	0.1	3200	392%	110	TC	10	1065
E D			2 0 700	179	TC	10	750	179	S	0.2	2400	292%	177	TC	10	1055
				177	TC	10	1055	176	S	0.2	6800	339%	166	TC	10	1180

\*TC—Theophylline sodium salicylate  
 PC—Phenylmethyl theophylline sodium salicylate  
 TM—Theophylline sodium salicylate  
 TBC—Theobromine sodium salicylate  
 TBS—Theobromine sodium salicylate  
 S—Salicylic acid  
 HgCl<sub>2</sub>—Bichloride of mercury  
 HgCN—Cyanide of mercury  
 AMP—Aminophylline  
 TPD—Theophylline  
 Purine—Purine sodium salicylate

"oral-purine-intravenous-mercury" regime was begun, and the facts must be taken into account in judging the results of the new procedure

The average twenty-four-hour output of urine for a test period, and the body weight at the end of this time constitute the basic levels. The average outputs are then recorded for several days during which a purine is given by mouth. Then the effect of adding an intravenous injection of usually 0.1 gm. salyrgan is noted and the purine alone is continued for several days. Reference to the table will show dramatic enhancement of the urinary excretion to more than 1000 per cent increase in several instances and usually a definite persistence of effects for several days in most cases. Although the experiments are comparatively few in number (12), there is such spectacular augmentation of urinary flow, agreeing so closely with our experience with intravenous administration of both drugs and our conceptions of the mechanisms of the two types of drug action that we accept them as complimentary evidence and feel no hesitancy in presenting them.

#### CONCLUSIONS

Clinical evidence seems to substantiate the idea that purine diuretics act primarily by increasing the glomerular filtration rate while the heavy metals accomplish their results principally by decreasing the tubular reabsorption.

Advantage was taken of these hypothetical considerations of the two different modes of action of the two types of diuretic drugs in the hope of getting better results by rational combination regimes.

Distinctly practical advances in the treatment of the vicious circle of edema in congestive heart failure are demonstrated.

The purines and heavy metal diuretics can be so combined as to (1) produce diuresis when either one of the drugs used alone in maximum dose has been ineffectual (2) as to accomplish the greatest possible diuresis in the shortest interval of time, (3) as to obtain a perfectly satisfactory diuresis by smaller and absolutely harmless doses which alone would prove inadequate.

Such complimentary effects, the result of combinations of the therapeutic action, even though hypothetically on different parts of the renal unit are, we believe, of distinct practical value.

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## PRIMARY CARCINOMA OF THE LIVER\*

### A CLINICOPATHOLOGIC STUDY

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THE liver is one of the organs in which secondary carcinoma occurs very frequently and primary carcinoma quite as infrequently. The malignant growths of the liver consist of epithelial tumors derived from the liver cells or the epithelial cells of the bile ducts, and mesenchymatous tumors originating either in the stroma or in the endothelium of the blood capillaries. The epithelial tumors are comparatively more frequent than the mesenchymatous ones. The purpose of this paper is to give a brief review of all the major contributions to this subject in the literature, and in this manner to establish as nearly as possible some idea as to its frequency. Further to call attention to some of the suggested conceptions concerning its histogenesis and finally to present analytical material, which is confined entirely to epithelial tumors, of 25 cases of primary carcinoma of the liver.

#### REVIEW OF THE LITERATURE

Prior to 1870, primary and metastatic carcinoma were not well differentiated. In 1876 Kelsch and Kiener<sup>1</sup> reported 2 cases, and at that time found only one other in the literature. Four more cases were added by Sabourin<sup>2</sup> in 1888 but the first thorough investigation was done by Hanot and Gilbert<sup>3</sup> later the same year. Von Hansemann<sup>4</sup> reported 258 cases of malignant tumor of the liver in 1890, 4 of which were primary.

Eggel<sup>5</sup> in his review of the literature up to 1901 reported 163 cases and one of his own. Among these only 117 were studied histologically. A summary of the reports up to 1916 by Winternitz<sup>6</sup> showed that the condition occurred in about 0.028 per cent of all necropsies. In a series of 3,700 postmortem examinations at the Johns Hopkins Hospital he found 3 cases. Goldzieher and von Bokay<sup>7</sup> in a series of 6,000 autopsies at the Pathological Institute of Budapest found 18 definite cases, an incidence of 0.3 per cent.

In Japan from the year 1904 to 1916 Yamano<sup>8</sup> collected a series of 57 cases found in 2,503 autopsies, an incidence of 2 per cent. Kika<sup>9</sup> in 1929 reported 110 cases found among 11,494 autopsies, and in a total of 20,185 autopsies in the Japanese literature since 1915, he found 223 cases, an incidence of 1.1 per cent.

Goldstein<sup>10</sup> in 1924 claims to have found, in the literature up to that time, about 250 cases of primary carcinoma of the liver as compared to 59 cases of sarcoma of the liver. Earlier in the same year Jaffe<sup>11</sup> reported a case of sarcoma and carcinoma occurring in the same liver. In the recent literature quite numerous reports of from one to several cases can be found. One obtains the impression in reviewing the subject that the condition is not as rare as is generally believed. Statistics of Orth<sup>12</sup> and von Hansemann<sup>4</sup> give about 0.5 per cent of all carcinomas as primary in the liver.

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It has been found in practically all ages and not uncommonly in children. Griffith<sup>12</sup> in 1915 compiled 57 cases in children ranging in age from one to sixteen years. In a baby girl of five weeks, Langmead<sup>11</sup> observed 8 purely white tumors of the liver which proved to be carcinomas arising from bile ducts. Wollstein and Mixsell<sup>13</sup> reported a case in a child of four months. Twenty three cases collected by Dransic<sup>14</sup> were under two and one half years of age. In 1929 Kilfoy and Terry<sup>1</sup> collected 43 cases in children from one to sixteen years and reported one case of their own. Among the 43 cases 12 were proved by autopsy, the remainder were doubtful.

Kaisner<sup>15</sup> concluded that primary carcinoma of the liver was most frequent in the fifth and sixth decades and this is supported by similar views from numerous investigators. As to sex incidence most writers agree that there is much more frequent occurrence in males. Geographically, those countries where liver infestations by flukes are common, report by far the highest incidence. From Japan, China, and South Africa evidences of this nature are quite numerous (Pirie,<sup>16</sup> Kikū,<sup>17</sup> Yamano<sup>18</sup>).

#### CLASSIFICATION

There are two great classes into which neoplasms can be divided, namely, the benign and the malignant. Typical examples of these can be differentiated with no great difficulty, but between these two extremes there are intermediate grades in which certainly the reverse is true. A study of liver tumors brings out very clearly the truth of this statement. Ewing<sup>20</sup> in his book on "Neoplastic Diseases" draws attention to several interesting factors which are necessarily understood. "The regenerative capacity of the liver as exhibited in the destructive and inflammatory conditions furnishes necessary standards in estimating neoplastic processes in this organ. Extensive regeneration of necrotic areas is observed after focal lesions produced by hemolytic agents, and especially in acute yellow atrophy of the liver. Prolonged cases of this disease may show many islands of new liver tissue springing from the surviving liver cells in the periphery of the lobules and producing irregular masses of deformed lobules which may present a very bizarre and almost adenomatous appearance."

In addition to reparatory and regenerative processes, hamartoma, originally described by Albrecht<sup>21</sup> in 1904, has been considered by some investigators to have a definite relationship to true neoplasms. The term hamartoma is applied to growths that arise by reason of disturbances of development, as is indicated by the literal meaning of the word, "developmental error." Albrecht states they are only abnormal mixtures of normal structural constituents of the organ in which they occur, be it of the number, arrangement or grade of differentiation, or in all three respects. The idea has been well expressed by Warthin,<sup>22</sup> a part of whose definition bears repeating. "From the true tumors or blastomas there must be carefully separated tumor like malformations or disturbances of development that show no autonomous excess of growth." He further intimates that such growths may form the basis for true neoplasms. Dylenforth<sup>23</sup> states that it seems plausible, in view of the fact that many kinds of neoplasms have corresponding hamartomas, which at times are difficult to distinguish from them, to consider the preexistence of such a condition in some of the cases of primary carcinoma. Thus, a hamartoma of any organ might easily be conceived as antecedent to neoplastic change, for it is known that these representatives of disturbance of tissue do form bases for malignant tumors. He suggests that they are rarely found possibly because they frequently go unrecognized, for it is not necessary to the premise that they become malignant.

He compares the gross and microscopic appearance of two cases of hamartoma of the liver with a case of early primary carcinoma of the liver and brings out some points in common

Concerning the histogenesis of these primary epithelial tumors Ewing states, "The growth of hepatoma from the hypertrophic liver cords has been observed by many writers. It has also been shown that there is a uniform gradation between nodular hyperplasia, multiple adenoma and multiple carcinoma. These gradations may even be observed in the same liver, so that as Mun has stated, there is no essential distinction between the comparatively benign and the atypical malignant forms of the tumor. During the transition the cells may retain the granular character of liver cells, while staining more intensely with basic dyes, or they may lose granules and pigment and assume the transparent, supposedly embryonal character described by Adler. Nuclear hypertrophy and hyperchromatism are very constant. Giant and multinucleated cells appear and mitoses and amitoses are frequent. Notable changes in the nuclei are often seen in the neighboring liver tissue, forming a feature of collateral hyperplasia. The first generation of tumor cells is usually large, later they may become smaller and atypical. The preservation and new growth of capillaries, whose endothelium resembles Kupffer cells, is a remarkable feature.

"Histologically there are two rather distinct varieties, one derived from the liver cells, the other from the bile ducts. As to the comparative incidence of these two varieties, there is nothing definitely settled among the writers who have committed themselves on this factor. Eggel found 32 per cent of primary carcinomas of the liver to be cholangiomas and the remaining 68 per cent hepatomas. Pepere reduced the proportion of cholangiomas to 14 per cent. Yamane<sup>8</sup> found 79 per cent to be of liver cell origin. In the literature definite reports of bile duct carcinomas are much less numerous than cases of liver cell carcinomas."

The liver cell tumors are subdivided into three types

1. A primary massive liver cell carcinoma. This type appears as a large single yellowish friable mass, often as big as a child's head, or occupying the whole right or left lobe. Small secondary tumors in the liver may be present. The consistency is usually soft and extensive liquefaction, necrosis and hemorrhage may produce a cystic appearance or rupture into the peritoneal cavity. The large veins are usually invaded and thrombosed. It is often associated with cirrhosis. Microscopically the structure presents wide variations, but always reveals at some points a definite resemblance to normal liver cells. In different portions four separate structures may be seen: (a) Trabecular adenocarcinoma composed of cords of very large granular acidophilic cells resembling large liver cells and separated by capillaries. Giant cells of very large dimensions are abundant in these areas. (b) Alveolar formation. These areas present compact groups of smaller granular epithelium sharply bounded by very numerous wide capillaries. (c) Peritheliomatous areas composed of capillaries surrounded by one or more rows of cuboidal granular cells. (d) Diffuse carcinoma. In many softened or necrotic areas all traces of the orderly arrangement of cells are lost, and the growth is composed of diffuse round polyhedral or spindle cells with strongly hyperchromatic nuclei.

These structural types cover most of those observed by other authors. It is evident that this tumor is a more rapidly growing, atypical and malignant form of the solitary adenoma.

2 Multiple liver cell carcinoma. In this group are included the highly malignant and rapidly growing tumors occurring in livers in which cirrhosis is absent or so slight as to be of secondary importance. There is, however, no sharp division between this group and the solitary massive carcinoma on the one hand and the multiple carcinoma following cirrhosis on the other. Here the liver is usually enlarged at times to very considerable dimensions and is the seat of multiple nodules and tumor masses which are grayish or bile stained or hemorrhagic and necrotic. Although the size of the tumor mass varies, it may be impossible to choose any one as a single primary focus. Many small nodules are doubtlessly secondary. Microscopically the structure varies greatly even in different portions of the same tumor and reproduces the more malignant features of the solitary carcinoma described above.

3 Carcinomatous cirrhosis. The tumor process appears to be a direct sequel of or essentially connected with cirrhosis. It shows an advanced stage of hyperplasia with atypical morphology, local aggressive properties and invasion of veins. The liver is usually contracted, but in some cases it is normal or somewhat increased in size. The surface presents multiple projecting yellowish or bile-stained nodules which on section may be found to represent a large part of the parenchyma. The nodules may be numerous, small and almost confluent, or larger, discrete and encapsulated. A portal cirrhosis is usually present, and many of the nodules are surrounded by connective tissue. Microscopic study shows extensive replacement of parenchyma by adenomatous and carcinomatous nodules. The tumor cells are of large size, forming cords, or the nuclei multiply actively and numerous smaller cells result. Peculiar forms of nuclear division, chiefly of the amitotic type are observed. Fatty degeneration may be prominent.

Biliary carcinoma has also been traced satisfactorily to the proliferating bile ducts. Precancerous changes are supplied by Milne and Yomagiwa in the form of angiocholitis proliferans. The multiple nodules must be referred to the universally distributed small ducts, while the solitary and cystic growths show some predilection for the hilus and subcapsular areas. This variety is less frequently associated with cirrhosis. The liver is usually enlarged. The dilated larger bile ducts may be visible in the gross. The tumor process affects most or all of the organ, and produces very numerous usually small firm nodules, which may become confluent. The extensive hemorrhages, necrosis, the bulky soft tumor masses, and prominent invasion of large veins, which characterizes the so-called hepatoma, are missing. A tendency toward cicatricial fibrosis has often been noted.

The structural features usually form a sharp contrast with those of liver cell carcinoma. The cells are cylindrical, high or low or cuboidal, resembling those of bile ducts. The cytoplasm is clear, lacking the granular acidophilic character of liver cells, while the nuclei are small and vesicular. Mitoses may be numerous but giant cells are rare. The stroma is abundant and reveals the desmoplastic property of true carcinoma, while in the hepatoma the stroma is usually composed only of capillaries, although a senilous type is described, which is very difficult to dis-



tinguish from a cholangioma. Most of the nodules are enclosed in capsules of connective tissue, and the gradation into hepatic parenchyma seen in hepatoma is usually missing. The outlying portal canals commonly exhibit marked proliferation of the bile ducts, as in biliary cirrhosis. The arrangement of the cells in small alveoli is characteristic and at once distinguishes most of the tumors from the coarsely trabecular neoplasms, derived from the liver cells. In the slower growths the alveoli are regular and adenomatous. In the more malignant cases they become smaller, more numerous and irregular.

There is a division of opinion among the writers, as to whether liver cells arise from proliferating bile ducts or bile ducts from the liver cells. Certain carcinomas of the liver present areas which exhibit the characteristics of a hepatoma, with other areas in the same liver which show the structure of a cholangioma. It is suggested that in such cases a mixed type of tumor results from a neoplastic change affecting both these elements simultaneously (Oisios<sup>4</sup>). The most detailed study of the histogenesis in primary malignant tumors of the liver is that reported by Oisios, in which several types of carcinoma as well as sarcoma are exhaustively discussed.

#### MATERIAL STUDIED

In the pathologic records of the Cook County Hospital from June, 1928, until April 1, 1932, 19 cases of primary carcinoma of the liver were found. During this stated period 3,846 autopsies were performed. In the department of surgical pathology 2 cases have been proved by biopsy, during the years 1930 and 1931, making a total of 21 cases from this institution. From the Research and Educational Hospitals associated with the University of Illinois, the records of 4 cases were found during the years 1930 and 1931. In this period 198 postmortems were recorded. This makes a total of 25 cases in 4,044 autopsies, showing an incidence of 0.56 per cent. The postmortem examinations and histologic study of the 21 cases from the Cook County Hospital were made by Dr. R. H. Jaffe.

#### CLINICAL FEATURES

The clinical course of a patient with primary carcinoma of the liver varies greatly. Several well defined clinical groups are observed. (1) Cases in which no symptoms are detected and the patient dies suddenly from hemorrhage, after an illness of a few days. (2) Instances in which latent carcinoma is found in patients succumbing to cirrhosis of the liver and other diseases. (3) Cases with the usual history of cirrhosis terminating rapidly with hepatic tumor, jaundice, ascites and cachexia. (4) Lastly the cases with the usual history of a malignant tumor pointing from the fist to the liver and developing in previously healthy subjects.

The ages of the patients ranged from twenty to seventy-three years, and about three fourths of the total number of cases were in the fifth and sixth decades. Twenty-three of the patients were males, the remaining 2 females. Comparative racial incidence showed 21 white and 4 colored individuals. The clinical courses varied in length from six weeks to eighteen months.

Conclusions can be drawn from Table I concerning the clinical picture and postmortem findings in each individual case. In these it is interesting to note that in no instance was the diagnosis definitely made clinically. In three the possibility

TABLE I

CLINICAL FINDINGS													POSTMORTEM FINDINGS																										
PAIN													METASTASES																										
CASES	RACE	SEX	AGE	ABDOMEN	BACK	SHOULDER	RAPID LOSS OF WEIGHT	WEAKNESS	NAUSEA AND VOMITING	ANOREXIA	PALPABLE MASS	LIVIDITY OF NILES	FLAYER	ANEMIA	DILATED ABDOMINAL VEINS	PALPABLE SPLEEN	DISTENTION	ALCOHOLISM	COURSE IN MONTHS	LIVER WEIGHT IN GRAMS	TYPE	CIRRHOSIS	ASCITES	GALLSTONES	RUPTURE INTO PERITONEAL CAVITY	PORTAL VEIN	LYMPH NODES	LUNGS	ADRENALS	PANCREATIC GLAND	PERITONEUM	RIBS	VEPTEBRAL	SKULL	ILIUM	CASES			
1	W	M	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	2400	LC*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1	
2	W	F	61	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8	1510	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2	
3	W	M	56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	5390	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	
4	W	M	57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7 1/2	4080	BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4		
5	W	M	57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	4810	BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5		
6	W	M	46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	12	3570	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6		
7	W	M	36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10	1070	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	7		
8	W	M	70	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1	5660	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8	
9	W	M	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	4380	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	9	
10	C	M	57	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	3460	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10	
11	W	M	73	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	1480	BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	11	
12	W	M	65	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	5020	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	12	
13	W	M	56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	1995	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13	
14	W	M	73	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	18	1990	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14	
15	W	M	19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16	2765	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15	
16	W	M	64	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	3280	BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16	
17	C	M	25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	2000	BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17	
18	W	M	69	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6	2460	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	18	
19	W	M	63	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	13	3620	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	19	
20	C	M	50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	4	1030	BD	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	20	
21	W	F	45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	6	1425	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21	
22	C	M	55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5	4020	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	22	
23	W	M	51	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	3860	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	23	
24	W	M	54	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	3860	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	24	
25	W	M	59	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	3860	LC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	25	
Totals																					9 20	3	2	7	10	9	5	1	2	1	2	1	2	1	2	1	2	1	Totals
Percentage																					16 80	12	8	28	40	36	20	4	8	4	8	4	8	4	8	4	8	4	Percentage
																					* LC liver cell BD, bile duct																		

\*LC liver cell BD, bile duct

was mentioned. The clinical diagnosis was secondary or metastatic carcinoma in 10 cases, cirrhosis of the liver in 8, gall bladder disease in 2, organic heart disease in 2, and kidney tumor, carcinoma of the pancreas, and subphrenic abscess diagnosed once each. It is generally conceded that the symptoms and findings of malignant tumors of the liver do not differ materially whether the growth be primary or secondary, carcinomatous or sarcomatous. In some instances there was no clinical evidence that the liver was affected. Pain, although it was by no means a constant symptom, was usually present at some period of the disease. It may be due to a stretching of the capsule or to a localized peritonitis. Occasionally it was more severe in the back than in the abdomen and infrequently referred to the shoulder. Among the 25 cases reported pain was present in 19, which to some degree and at some time was in the abdomen. In 6 it was felt principally in the lumbar region and two of the patients complained of pain in the shoulder. In these latter cases a solitary massive type of tumor was found in one lobe, which in both instances involved the diaphragm. Progressive loss of weight and strength was noted in all cases, but particularly was this true in the terminal stage. Jaundice occurred in 18 of the patients and in almost all cases was from mild to moderate in degree.

As nearly as possible an attempt was made to determine what symptom was first noticed by each patient. Seven complained of abdominal pain, 3 of lumbar pain and one of pain in the left shoulder. Of the remaining patients 5 first noticed a painless enlargement of the abdomen, 3 an anorexia, 3 a weakness, while dyspnea, edema of the lower extremities, and constipation were first apparent in one case each.

Progressive enlargement of the liver is perhaps the most important physical sign. A mass in the abdomen was palpated in 23 of the 25 patients. In 21 of these hepatomegaly was an outstanding finding. In 2 the liver enlargement was doubtful. The enlargement in one of these cases was thought to be a pancreatic tumor and in the other a renal tumor. It is also interesting to note that definite clinical signs of ascites was elicited in 18 patients or 72 per cent. Fever was found in 11 cases or 44 per cent. Eight of the patients showed dilated superficial abdominal veins or 32 per cent.

After the appearance of a malignant tumor of the liver, life is seldom prolonged over four months. Recovery is rare, but Keen<sup>25</sup> and Yeoman<sup>26</sup> reported successful excisions, some of which appeared to have recovered permanently. Six out of their 16 patients operated upon were alive and well from three to seven years postoperatively.

#### ANATOMICAL FEATURES

Grossly the livers varied in weight from 1030 to 5660 gm. As to consistency they were firm to hard, except when necrotic, and often showed thickened capsules. Only occasionally was there a decrease in size from the normal. Of the 23 cases which came to autopsy 4 livers weighed less than 1500 gm. The average was about 3 kilograms. The large solitary nodes, particularly, were often prone to hemorrhagic and necrotic changes. In two instances, there was a rupture of such nodes into the peritoneal cavity with fatal hemorrhage. On section, the nodes often presented alternating grayish pink to yellowish brown soft masses, penetrated here and there by white fibrous strands. Only a narrow edge of the liver parenchyma

remained of the lobe involved. In the multiple nodular type the liver substance was uniformly invaded by more or less sharply circumscribed reddish gray or bile stained nodules, varying from pinhead size to 6 cm. in diameter. In the carcinomatous cirrhotic type the desmoplastic reaction was more apparent, but there are transitions between these two latter types, making it difficult to differentiate them. Occasionally a liver will resemble externally an atrophic cirrhosis.

Six of the livers showed large solitary nodes with from few to numerous small intrahepatic nodules. Thirteen cases showed multiple nodular carcinoma and carcinomatous cirrhosis. The remaining 6 were cholangiomas. In these the nodules were usually ovoid and presented a rather colorful picture, the color ranging from whitish gray to brownish pink, sometimes bile stained, while the intervening liver tissue was brown, occasionally interspersed with yellow or yellowish green.

#### MICROSCOPIC FEATURES

The microscopic picture of the liver cell carcinomas reveals the tumor cells to be arranged in a very irregular fashion, often in interlacing strands, showing great variation in appearance. There may be some increase in the periportal connective tissue, which surrounds irregular islands of tumor cells, arranged in cords, but without grouping around a central vein. Some of the islands may present intercellular bile capillaries which are distended and filled by bile casts. Much bile pigment is occasionally found inside the liver cells. There are areas in which the cells are much increased in size with large hyperchromatic nuclei, undergoing direct division. Other areas are composed of irregular liver cells which form wavy bands and glandlike tubular structures, which not infrequently look strikingly like adenocarcinoma. The cytoplasm of these cells is homogeneous and oxyphilic. The nuclei are relatively small, round and rich in chromatin. In these areas no bile pigment is found. Many of the nodules show a tendency toward regressive changes and some of them are completely necrotic. Some nodes are composed of branched and anastomosing cellular cords, which are separated by thin strands of fibrillar connective tissue and capillaries. The cells which form the cords are polyhedral with an ample homogeneous slightly basophilic cytoplasm and round or oval nuclei. In the periphery of the nodes the tumor cells seem to pass into the cords of the adjacent liver tissue. By decreasing in size, assuming the basophilic coloration and enlargement of the nuclei the liver cells gradually become transformed into tumor cells. At times various sized and shaped cellular areas are separated by bands and regions of intercommunicating trabeculae of liver cells. The former areas are composed of finely granular light staining cytoplasm and large vesicular nuclei containing several oxyphilic nucleoli. In other cases they are seen in an alveolar arrangement, the alveoli being composed of round and polyhedral cells with distinct oxyphilic cytoplasm and round nuclei having a finely granular chromatin network. There are numerous mitotic figures, some being huge and markedly irregular. Giant and multinucleated cells are quite frequently seen. In the lumen of the portal capillaries and of the branches of the sublobular veins, tumor cells can be found. In the cells of the adjacent liver parenchyma much fat in the form of medium sized and large droplets is present. There are occasional erythrocytic extravasations.

The structure of the tumors arising from the bile ducts may also resemble that of an adenocarcinoma very frequently. The character of the tumor is tubular, and in the youngest areas is similar to proliferating bile ducts. There are narrow branched anastomosing tubuli lined by irregular cuboidal and, in some cases, columnar epithelium. The tubuli in a few instances become widened, and then lining reveals a tendency to form papillary infoldings. The papillae fuse together and thus finally the tubules result in a cell-filled alveolus. In the center of the alveoli there are at times extensive regressive changes. The cells are of irregular shape and size, and the anaplasia culminates into single huge elements with several bizarre nuclei. There is also a very abundant sclerotic stroma. In other cases the tumor is composed of dense fibrous tissue, in which single and groups of cells with large hyperchromatic and irregularly shaped nuclei and a relatively ample acidophilic cytoplasm are seen. Only small islands of liver tissue remain in most instances. The tumor nodules in many cases seem to be surrounded by fibrous connective tissue which separate them from the surrounding liver structure, whereas, in the so-called hepatomas there is rather a gradual merging of the tumor with the liver cells. Occasionally the picture may resemble very strikingly that of a hepatoma.

The frequency of metastatic growths is not great. They are principally hematogenous, although extensions to adjacent lymph glands and peritoneum is undoubtedly lymphogenous. Five of the cases reported showed no metastatic growths, and in four instances they were limited to branches of the portal vein. In the remainder of the cases the secondary growths were distributed in the lungs, adjacent lymph nodes, peritoneum, adrenal glands, parotid gland, meninges, ribs, spine, skull, and iliac bones. Table I shows the numerical distribution of the metastases in these various places. Secondary growths were found in the lungs in 9 of the cases and extension to the neighboring lymph glands was present in 10. Curiously, the adrenal glands showed metastases in 5 instances and in all cases the right gland was affected. In 3 of the cases bone metastases were discovered. Frequently the distant metastases of the liver cell carcinoma exhibited liver cell cords, Kupffer cells, capillaries, and bile secretion. This is important in that it offers proof of the origin from liver cells.

Of 163 cases tabulated by Eggel,<sup>5</sup> 46 were free from definite extensions, 50 showed growths limited to the portal or hepatic branches, 30 gave metastases to the lungs and thorax, 18 in regional lymph nodes only, and 9 secondary tumors were distributed in the colon, pancreas, ovary, kidney, omentum, thyroid, cranium, and brain. Moon,<sup>27</sup> in an article on bone metastases, reports the following sites. In the neck of the femur with spontaneous fracture, in the vertebrae, ribs, sternum and pelvic bones. Gadiat<sup>28</sup> reports a case with a secondary growth in the corpus cavernosum, producing a false priapism.

#### ASSOCIATED PATHOLOGY

There is little doubt that cirrhosis and the factors that lead to it cause degeneration followed by regeneration which may become excessive and neoplastic. It is one of the chief predisposing factors, occurring in about 85 per cent of the liver cell carcinomas and 50 per cent of the biliary duct tumors, according to Ewing.<sup>20</sup>

In our experience, however, the percentage is much less. Cirrhosis was found during postmortem examination in 9 cases of hepatomas and in none of the cases of cholangioma. Two of the cases were diagnosed by biopsy, and whether cirrhosis was present or not could not accurately be determined. This makes an incidence of 39 per cent of all cases which is somewhat less than the reports of other writers.

Hemachiomatosis associated with primary carcinoma of the liver has been reported in 14 instances or 9 per cent. Rosenthal<sup>9</sup> concludes that all the cases of hemachiomatosis presented cirrhosis of the liver and that this relation of these two conditions does not predispose to carcinoma more frequently than cirrhosis of the liver alone.

#### SUMMARY

In conclusion it suffices to say that in reviewing the literature it would seem that primary carcinoma of the liver is less rare than it has generally been believed. Certainly the highest incidence is reported in the Orient (2 per cent). The 25 cases reported here show an incidence of 0.56 per cent of all necropsies done at the Cook County and Research and Educational Hospitals, and 4.9 per cent of all malignant tumors. Relatively few cases of cirrhosis of the liver associated with primary hepatic carcinoma were found in this series of cases (39 per cent) which might indicate that perhaps it is not as important a predisposing factor as has been previously reported. Up until the present time, there has been little attempt to show that there are factors other than cirrhosis which are of importance in the origin of primary carcinoma of the liver. Evidence supporting the relationship which seems to exist between hamatoma of the liver and primary carcinoma of that organ is to a large extent convincing. It is urged that these malformations or developmental errors be sought for in the liver and studied with the thought in mind that many in the past may have gone unrecognized. In the mixed variety of tumors where the features of both the liver cell and bile duct carcinomas are found in the same liver, it is suggested that this is due to a neoplastic change affecting both these elements simultaneously. In most of the cases only one is affected resulting in a typical liver cell or bile duct variety. Attention is called to the difficulty in making the diagnosis of primary carcinoma of the liver clinically. Analytical data including a summary of the clinical histories, postmortem and histologic findings of 25 cases of primary carcinoma of the liver are reported.

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SWELLING OF THE EXTERNAL GENITALIA IN CASTRATED  
FEMALE BABOONS AFTER ORAL AND HYPODERMIC  
TREATMENT WITH FEMALE FOLLICULAR SEX  
HORMONE\*

W. SCHOELLER, M. DOHRN, AND W. HOHLWEG

THE question of a definite follicular hormone dosage is still a problem. There has been considerable difference of opinion with regard to the size of the dose of follicular hormone in rodents. The view was held by many that the same dose which produces estrus, as measured by the Allen and Doisy test, is also the physiologic dose required to produce the complete phenomena of heat in mice or rats. Paikes<sup>1</sup> was the first to show that this is not the case. Normally, in sexually mature female rats, the vaginal changes occurring during the period of heat are also accompanied by hyperemia and increase in size of the uterus. An Allen and Doisy rat unit, while it admittedly produces an increase in the epithelialization and cornification of the vaginal mucous membrane, does not, however, suffice to cause a definite change in the uterus. Furthermore, the psychologic condition characteristic of heat is also only obtainable through the administration of larger doses than the Allen-Doisy unit. These results obtained by Paikes were confirmed in experiments carried out by Hohlweg and Dohrn.<sup>2</sup> They ascertained that the daily administration to adult, castrated female rats of a dose of follicular hormone which was capable of producing permanent estrus (e.g.,  $\frac{1}{2}$  to 1 rat unit daily) did not, however, suffice to prevent the castration changes in the anterior pituitary. To achieve this object it was necessary to give 3 to 6 rat units daily. From this work it will be seen that a daily dose which produces permanent estrus in mature female rats, and might thus be considered an overdose, is nevertheless insufficient to prevent another defect of castration and actually must therefore represent either too small a dose or an unsuitably spaced dosage. If then the drawing up of a scheme of physiologic hormone treatment for a species of animals such as the rat, about which so much is known, presents such difficulties, it will readily be seen that the determination of the physiologic dose of the hormone in human beings is a task of some magnitude.

For this reason we had already conducted earlier experiments on monkeys, which more readily allow of conclusions as to conditions in man. In these earlier researches<sup>3</sup> we were mainly concerned with demonstrating the periodical effect of the follicular hormone on female monkeys. We found that in the infantile female macacus rhesus 2000 rat units administered over a period of a month in the form of pulverized tablets are sufficient to procure the full development of the previously infantile genital organs. The vagina,

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cervix, uterus, and mammae of the infantile animals show the same degree of development as is normally observed in mature females of the species during heat, i.e., at the time when the graafian follicle bursts and the maximum follicular hormone effect is observed. A comparison of the illustrations shown in the above mentioned treatise with those reproduced in the publication of Zuckermann and Parkes on female baboons clearly demonstrates this fact.

We conducted further tests on female baboons as in these animals an external change, i.e., swelling of the vulva, offers a method of estimating the effect of the follicular hormone. Furthermore baboons are more nearly related to human beings both in size and in the character of the sexual cycle than the macacus rhesus. The female baboon shows a fairly regular menstrual cycle which, just as in women, is accompanied by monthly bleeding. The normal cycle lasts on an average thirty days. The characteristic swelling in the genital region and perineum reaches its maximum at the time of ovulation. The swelling increases in size for seven to eight days, maintains its maximum degree for a few days and then rapidly declines (Zuckermann and Parkes,<sup>4</sup> Blotzovogel.)

According to Zuckermann and Parkes the bleeding takes place twelve to fourteen days after the end of the period of maximum swelling. In castrated animals swelling does not occur. The appearance and disappearance of these typical signs of heat is therefore dependent upon the ovary and coincides almost exactly with the follicular phase. One could therefore assume that this change was produced by the follicular hormone and in actual fact Parkes and Zuckermann succeeded in reproducing this swelling artificially in castrated females by injection of the follicular hormone. Since these two English workers had confined their experiments to parenteral administration of the hormone, we resolved to investigate the question as to what peroral and intramuscular doses were necessary to reproduce swelling of the external genitalia in castrated female baboons.

*Experiments on Peroral Administration*—In the first experiments, in order to make sure of obtaining a positive effect we gave the animal L (11.2 kilos) the high daily dose of 6 000 rat units progynon by mouth. We planned to continue the treatment for eight to ten days, in accordance with physiologic conditions. One gram of an extract from the urine of a pregnant animal containing 6 000 rat units was ground into powder with sugar and emulsified with 30 per cent alcohol. This mixture was poured into the animal's gullet once a day and followed by a drink of water so that for all practical purposes the entire quantity was taken. As early as the fourth day reddening of the nipples and genital region were already noticeable and the typical swelling quickly followed. At the end of nine days, during which the animal had received a total of 54 000 rat units, we suspended the treatment. The swelling reached its height on the second day following the last dose and was very marked.

In view of the definite reaction obtained in the first experiment, we conducted a second trial with only one-tenth of the previous dose, i.e., 600 rat units daily, administering this amount for eight days. The animal K (12

kilos) was employed and Figs 1-A, 1-B and 2-A, 2-B show that in this case also a definite effect was obtained with a total dose of 4 800 rat units

One month after the subsidence of the swelling the same animal was given 100 rat units daily for eight days and only a slight reaction was observed. From these three preliminary experiments we concluded that during an eight-day treatment about 6 000 rat units are necessary perorally in order to reproduce definite genital swellings in castrated female baboons, provided that a product is used which is active perorally. For the subsequent tests we employed progynon<sup>\*</sup> tablets. In the fourth experiment the tablets were pulverized and administered in water. The animal J (weight 12 kilos) did not react to one and one-half tablets of 200 rat units given daily for eight days

Fig. 1-A

Fig. 1-B

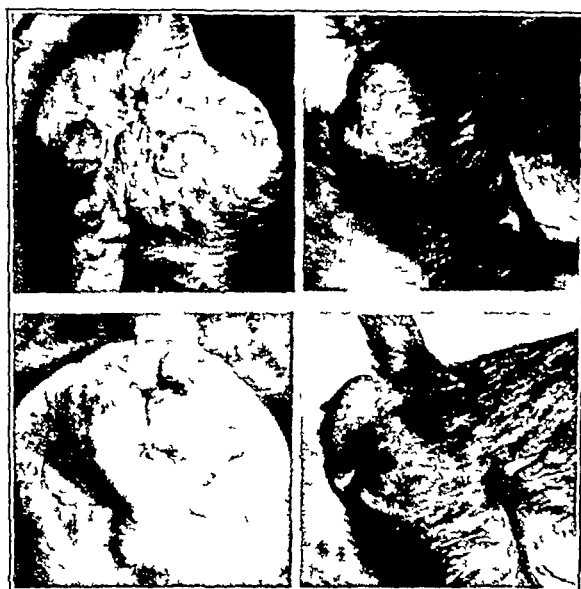


Fig. 2-A

Fig. 2-B

while the animal K showed a good reaction with double this dose. In the fifth experiment we gave the tablets whole, and in this form, they proved equally effective.

The results of the sixth experiment were very interesting indeed. We had prepared tablets of 600 rat units each, and one of these tablets was administered daily for eight days to the three animals J, K, and E (weight 11 kilograms each). In this experiment the different reactive capacity of the three individual animals of nearly similar size was clearly demonstrated. J did not react at all, while K showed a moderate effect, and in E the results were very marked, as Figs 3-A, 3-B, and 4-A, 4-B show.

In the seventh experiment the animal J, which had not reacted to  $8 \times 1$

<sup>\*</sup>Progynon is a preparation of the standardized female follicular sex hormone in tablet and ampule form. It is not reduced to the crystalline state as is the case in other preparations. The tablets contain certain lipoids of the placenta and ovaries in order to facilitate their absorption. The absorption of the tablets is on a ratio of one to five, one rat unit in five being absorbed when taken by mouth.

tablets of 600 rat units, showed marked swelling of the external genitalia after 2 tablets daily

From these experiments it would appear that if our tablets are used about 6 000 to 8 000 rat units are necessary in order to produce the characteristic genital swellings in castrated female baboons provided the total dosage is spread over eight days

*Experiments on Intramuscular Administration*—Our trials with intramuscular administration of crystalline follicular hormone and its benzoate also gave interesting results. Butenandt<sup>10</sup> showed that progynon benzoate is capable of producing prolonged estrus in rats, and we were able to check his observations with a preparation made in our laboratory by Dr. Hildebrandt

Fig 3-A

Fig 3-B



Fig 4-A

Fig 4-B

With a single dose of 0.25 mg progynon benzoate estrus lasting on an average thirty days was produced in castrated female rats. Since the female ape has a longer follicular phase than the female rat, a longer excretion of the follicular hormone must be reckoned with on physiologic grounds. It was therefore to be expected that in female baboons a more marked difference in the effectiveness of the same dose would be observed, according to whether this was administered in a single dose or in divided doses and similarly according to whether a single dose of the free hormone or its ester was given. In the eighth experiment the animals E and M received an intramuscular injection of 5 mg of crystalline follicular hormone<sup>11</sup> dissolved in 1 c.c. of oil

<sup>10</sup> Crystals prepared from the urine of pregnant mares, melting point 24°. C. Standardized on rats. The preparation dissolved in oil and injected in three portions spread over 1 day. Result: 100 000 rat units per gram. Judged by logical activity and the nature of the raw material this preparation was fairly pure progynon the strength of which is estimated by Butenandt at 300 000 to 100 000 rat units per gram.

Fig 5-A

Fig 5-B



Fig 6-A

Fig 6-B

Fig 7-A

Fig 7-B



Fig 8-A

Fig 8-B

Five mg corresponded to 2 000 iat units. The animals J and K were injected with 6.925 mg of progynon benzoate† dissolved in 1 cc of oil.

†Progynon benzoate with a melting point of 215° to 216° C prepared from crystals obtained in the manner above described.

TABLE I  
SUMMARY OF EXPERIMENTS ON THE PERORAL ADMINISTRATION OF PROGYNON

EXPERIMENT	ANIMAL	PREPARATION	DURATION OF TREATMENT	DOSAGE IN RAT UNITS	RESULT
I	L	Progyron oil in alcoholic aqueous sugar solution	9 days	Daily 6 000 Total 54 000	Pronounced swelling
II	K	Progyron oil in alcoholic aqueous sugar solution	8 days	Daily 6 600 Total 54 000	Pronounced swelling
III	K	Progyron oil in alcoholic aqueous sugar solution	8 days	Daily 0 100 Total 0 800	Only very slight swelling
IV	K	Progyron tablets (200 R U) suspended in water	8 days	Daily 0 600 Total 4 800	Pronounced swelling
	J	Progyron tablets (200 R U) suspended in water	8 days	Daily 0 300 Total 2 400	No swelling
V	K	Progyron tablets (200 R U) given whole	8 days	Daily 0 600 Total 4 800	Medium swelling
VI	J	Progyron tablets (600 R U) given whole	8 days	Daily 0 600 Total 4 800	No swelling
	K	Progyron tablets (600 R U) given whole	8 days	Daily 0 600 Total 4 800	Medium swelling
	E	Progyron tablets (600 R U) given whole	8 days	Daily 0 600 Total 4 800	Pronounced swelling
VII	J	Progyron tablets (600 R U) given whole	8 days	Daily 1 200 Total 9 600	Pronounced swelling

According to the standardization on rats, this dose of the benzoate corresponded in activity to the dose of free hormone given to the first two animals. The animals E and M, which were injected with the crystalline hormone, showed a very weak reaction on the fourth day after the injection and this rapidly disappeared. In the other two monkeys who received the benzoate, the swelling also commenced on the fourth day but increased considerably up to the seventh day, and remained constant for two or three days. Figs 5-A and 5-B to 8-A and 8-B, which were all taken on the seventh day after injection, show the different effects of the free and the esterified hormone.

TABLE II  
SUMMARY OF EXPERIMENTS ON THE INTRAMUSCULAR ADMINISTRATION OF PROGYNON

EXPERIMENT	ANIMAL	PREPARATION	DURATION OF TREATMENT	DOSAGE IN RAT UNITS	RESULT
VIII	E	Progyron cryst 5 mg in 1 cc oil	1 day	2 000	Slight swelling
	M	Progyron cryst 5 mg in 1 cc oil	1 day	2 000	Very slight swelling
	J	Progyron benzoate 69 mg in 1 cc oil	1 day	2 000	Pronounced swelling
	K	Progyron benzoate 69 mg in 1 cc oil	1 day	2 000	Pronounced swelling
IX	E	Progyron cryst 25 mg in 1 cc oil	8 days	Daily 0 250 Total 2 000	Pronounced swelling
	M	Progyron cryst 25 mg in 1 cc oil	8 days	Daily 0 250 Total 2 000	Pronounced swelling
	J	Progyron cryst 25 mg in 1 cc oil	8 days	Daily 0 500 Total 4 000	Pronounced swelling
	K	Progyron cryst 25 mg in 1 cc oil	8 days	Daily 0 500 Total 4 000	Pronounced swelling

The fact that the ineffectiveness of the 5 mg of crystalline hormone was solely due to its administration in a single dose was clearly shown in the ninth experiment. In this experiment the animals E and M received the same dose as in experiment 8 but on this occasion the 5 mg of crystalline follicular



Fig 9-A

Fig 9-B

hormone were spread over eight days. Both animals showed swelling of the external genitalia and Figs 9-A and 9-B show the definite effect of the divided dosage (cf Figs 5-A and 5-B).

#### SUMMARY

The experiments show that with the peroral administration of 6 000 to 8 000 rat units of progynon spread over a period of eight days, the genital swellings characteristic of heat can be reproduced in the castrated female baboon. With subcutaneous injection of crystalline follicular hormone dissolved in oil 2 000 rat units spread over a period of eight days produced the same effect, while the same dose given in a single injection is inactive. If, however, progynon benzoate is used, a single injection of 2 000 rat units is sufficient to produce a definite reaction. Our investigations once again demonstrate that with a suitably chosen product and tablets of a particular composition, the peroral dose is only four to five times greater than the parenteral one.

In further researches which are at present in progress, we are endeavoring to bring about artificially the entire sexual cycle, including menstruation, in castrated female baboons, by preliminary treatment with follicular hormone, followed by the administration of corpus luteum hormone. The knowledge acquired in these experiments should then be sufficient to bring about menstruation in castrated women by combined treatment with these two hormones.

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## THE ANTIDOTAL EFFECT OF SODIUM AMYTAL IN STRYCHNINE POISONING\*

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DAWSON and Taft<sup>1</sup> and Haggard and Gincenburg<sup>2</sup> observed that several barbituric acid derivatives are antidotes in strychnine poisoning. Swanson<sup>3</sup> administered intravenously single equivalent effective doses of sodium amytal and pentobarbital sodium in rabbits poisoned with strychnine. In these experiments sodium amytal antidoted  $6\frac{2}{3}\%$  times the MLD of strychnine and pentobarbital sodium  $4\frac{1}{3}\%$  times the MLD of strychnine. Dawson and Taft<sup>1</sup> and Barlow<sup>4</sup> reported the injection of small effective doses of barbituric acid derivatives repeated at intervals as judged by the recurrence of strychnine convulsions. Clinically, in 1929 Zetias and McCallum<sup>5</sup> found that sodium amytal when injected intravenously in single and repeated doses was an extremely effective and life saving antagonist to strychnine poisoning. At the same time, rabbits poisoned with 2.5, 5, and 10 times the MLD of strychnine were saved by single and repeated intravenous doses of sodium amytal (unpublished data).

This report is a continuation of the study of antidotal action of repeated small effective doses of sodium amytal in rabbits poisoned with strychnine.

Generally, it is agreed that 0.6 mg per kg of rabbit injected subcutaneously is the fatal dose of strychnine sulphate. In these experiments a 2 per cent solution of strychnine sulphate was used. Five and 10 per cent solutions of sodium amytal were used. The rabbit weights varied from 2.1 to 4.34 kg. Doses of 25 mg and 12.5 mg per kg of sodium amytal were injected intravenously immediately following or during the first convulsion and repeated at intervals as required by the recurrence or severity of the convulsions. Injections of sublethal doses of sodium amytal made too rapidly or too frequently may result in respiratory failure and cardiac disturbance.

As shown in Table I, 12 mg per kg or 20 times the MLD of strychnine require an average of 112.5 mg per kg of sodium amytal to save 4 out of 5 rabbits. With 15 mg per kg or 25 times the MLD of strychnine, it requires an average of 121.5 mg per kg of sodium amytal to save 7 out of 10 rabbits. For 18 mg per kg or 30 times the MLD of strychnine, an average of 137.5 mg per kg of sodium amytal is essential to save 6 out of 10 rabbits. Two of the surviving rabbits received artificial respiration at intervals during the treatment. Twenty-one mg per kg or 35 times the MLD of strychnine require 143 mg per kg of sodium amytal plus artificial respiration to recover 5 out of 10 rabbits. Five rabbits injected with 24 mg per kg or 40 times the MLD of strychnine fail to recover with 152.5 mg per kg of sodium amytal plus artificial respiration. Strychnine in such large doses probably exerts an additive depressive action with sodium amytal.

\*From the Lilly Research Laboratories, Eli Lilly and Company.  
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TABLE I

ANTIDOTAL EFFECT OF SODIUM AMYTAL IN RABBITS POISONED WITH SUBCUTANEOUS INJECTIONS OF STRYCHNINE SULPHATE

NUMBER OF RABBITS IN SERIES	STRYCHNINE SULPHATE MG PER KG	NUMBER OF LETHAL DOSES OF STRYCHNINE SULPHATE	SODIUM AMYTAL MG PER KG	NUMBER DIED	NUMBER SURVIVED
5	12	20	112.5	1	4
10	15	25	121.5	3	7
10	18	30	137.5	4	6
10	21	35	143.0	5	5
5	24	40	152.5	5	0

This shows that sodium amytal injected intravenously is antidotal to strychnine in 60 per cent of the rabbits poisoned with 30 times the MLD of strychnine. Fifty per cent of the rabbits receiving 35 times the MLD of strychnine recover with sodium amytal, together with the use of artificial respiration. With sodium amytal, it requires more to antidote equal amounts of strychnine than with pentobarbital sodium.<sup>4</sup> This difference in amount of sodium amytal is not twice that of pentobarbital sodium as would be expected, although the intravenous MLD of pentobarbital sodium in rabbits is 40 mg per kilogram and that of sodium amytal 80 mg per kilogram (unpublished data). The real difference apparently is the duration of action. With equal effective doses of sodium amytal, the duration of action is obviously longer than that of pentobarbital sodium. Thus during the critical period of strychnine poisoning, sodium amytal is less frequently required.

#### CONCLUSIONS

Sodium amytal by vein in small repeated doses antidotes 20, 25, 30, and up to 35 times the subcutaneous MLD of strychnine sulphate in rabbits.

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## PRIMARY SARCOMA OF HEART\*

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THE heart is peculiarly immune to neoplasm, and particularly to primary tumor. It is therefore deemed noteworthy to report a case which came to autopsy at the City Morgue in Brooklyn, New York, Kings County Hospital, a case of sudden death upon the street, and reported to the Medical Examiner's Office in that Borough of New York City.

The completion of a routine autopsy performed on this case proved it to be one of unique interest. Investigation of the literature on primary cardiac neoplasm has instigated this report. Through the courtesy of Dr. Manuel E. Marten, Deputy Chief Medical Examiner of Brooklyn, we are enabled to report and place this case on record.

This is a case (W. P.) of a well-developed, muscular negro, aged fifteen, 5 feet tall and weighing about 135 pounds.

*Family History*—The father was a sailor and apparently in good health, the mother was also living and well. She admitted only one pregnancy, that of the deceased. Delivery was normal, and at the time of birth no abnormalities were noted.

*Physical History*—He was subject to frequent colds and sore throats. Syphilis and gonorrhea were denied, had no exanthemas, had been subject to chronic bronchitis, had had no operations or hospital treatment, no addiction to alcohol or drugs. Appetite was good, slept well, mode of life was within normal limits. Apparently, no history of neurologic manifestations or systemic disorders of any nature.

*Present Illness*—He had been employed as an errand boy, attended night school and participated in gymnastics, and occasionally attended social functions such as dances. On the night of his death, while on the way to a party, he complained to a companion of feeling ill and started for his home, when he collapsed in the street from sudden illness. An ambulance was summoned, and upon its arrival he was pronounced dead. The body was removed to the City Morgue at Kings County Hospital for determination of cause of death, and necropsy showed cardiac neoplasm. Upon further investigation it was ascertained that about a year and one half before his death he began to complain of dyspnea and cardiac palpitation on exertion, and was treated by a physician as a cardiac. No history of cardiac symptoms is available that may have existed prior to his having come under the doctor's care. His health having been uniformly good prior to his cardiac history naturally curtails the clinical history in this case.

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\*From the Pathological Laboratory of Kings County Hospital, William W. Hala, M.D., Director.

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At necropsy the following findings were established

Head Scalp and skull negative

Brain Subpial congestion and edema

Thorax Heart weighed 1325 gm There was a tumor-like mass in the right auricle, filling the entire chamber and consisting of soft, fat-like material interlaced with coarse fibrous strands The mass was  $6\frac{1}{4}$  inches in its greater diameter and 4 inches in the lesser and correspondingly distended the auricle (Figs 1 and 2) The wall of the auricle averaged  $\frac{1}{4}$  inch in thickness, and



Fig 1—This picture illustrates the enormous size of the right auricle distended by the tumor within it 1 Right auricle 2 The rest of the heart including the left auricle and both ventricles 3 Right lung } Points of rupture which occasioned the sudden death and the patient.

showed at its upper border 2 ruptures one  $1\frac{1}{4}$  inches in diameter and the other  $\frac{3}{4}$  inch Both openings were covered with blood clot The left auricle appeared normal The combined width of the ventricles was  $3\frac{3}{4}$  inches by  $3\frac{1}{2}$  inches, and the chambers were normal The musculature was of good quality, and the valves and the orifices were grossly normal The aorta was normal except for numerous atheromatous plaques on the intima

Right Lung Weighed 480 gm, was displaced by the enlarged auricle and compressed to about three fifths the normal size, dissection of the lung showed

atelectasis of the middle lobe and slight exudation of frothy fluid from the upper and lower lobes

**Left Lung** Weighed 510 gm, on section, frothy fluid was expressed from the cut surfaces

**Right Pleural Cavity** Contained about 1½ quarts of fluid and blood clot. The right auricular wall was adherent to the pericardium at the points of rupture, and the fluid and clotted blood had escaped through the pericardium into the right pleural cavity



Fig 2—Anterior view of the heart and the enormous auricular tumor. The growth in size greatly exceeds the rest of the heart. 1 Right auricle 2 The rest of the heart 3 Right lung 4 Trachea 5 Points of rupture

**Abdomen** Liver Weighed 1300 gm On section showed chronic passive congestion only

**Spleen** Weighed 40 gm The capsule was tense and slate blue in color. On section, the pulp was firm and the follicles prominent

**Kidneys** Combined weight 250 gm The surfaces were reddish brown in color and engorged. No gross pathology was found on section. The ureters and urinary bladder showed no changes

The pancreas, adrenals, gastrointestinal tract, and the remaining viscera were grossly normal

Examination of the bones showed no gross changes

The histologic examination of sections taken from the auricular tumor revealed a histoid neoplasm composed exclusively of small round cells of lymphocytic morphology. There were areas of necrosis which accounted for the peculiar yellowish (lipoidal) appearance of the tumor, and which on gross examination caused a provisional diagnosis of liposarcoma.

Primary tumors of the heart are rare. Usually cases are demonstrable at autopsy only, since the diversity of clinical manifestations, involving, as they do, a variety of lesions, give no specific indication of cardiac tumor. It may therefore be stated that neoplasms of the heart are of greater interest to the pathologist because of their objectivity, than to the clinician. On the other

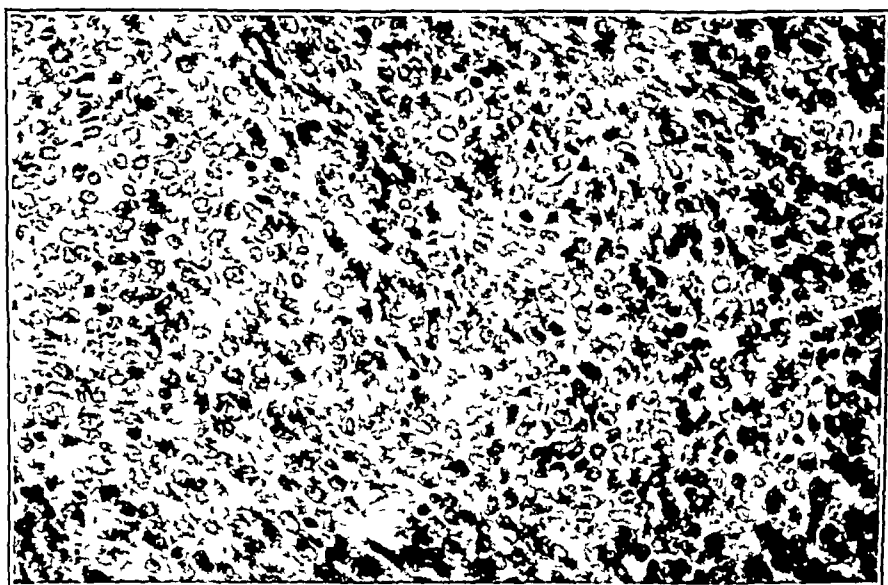


Fig 3—The histologic architecture of the tumor is well shown in this photograph. It is entirely histoid and consists entirely of small round sarcoma cells within a delicate meshwork of reticulum.

hand, review of the literature would seem to indicate the possibility of identification of primary cardiac tumor during life, although nothing specific appears to be adduced, to substantiate this.

Metastatic involvement is of course of far more frequent incidence, and its existence in the heart is inferred because of the determination of a primary focus elsewhere. The origin of such metastases has been reported from melanotic sarcoma of eye, and cancers of the breast, mediastinum, stomach, adrenals, uterus, and penis. The majority of these secondary tumors of the heart are carcinomatous in type rather than sarcomatous.

In the premicroscopic days Morgagni,<sup>1</sup> in 1762, described multiple polypoid tumors of the heart, but as to whether these were intramural thromboses or true neoplasms is, of course, speculative. The first authentic demonstration of primary cardiac tumor was that of chondroma by Albers,<sup>2</sup> in 1835, and di-

agnosed histologically as fibroma. The first malignant connective tissue tumor was reported by Bodenheimer<sup>2</sup> in 1865.

More than 100 cases have been reported of primary tumor of the heart, but many of these have been rejected by histopathologic findings and classed as metastases or pseudotumors. Tedeschi<sup>4</sup> reviewed 86 cases, including 3 of his own, many of which were secondary tumors.

Beirhenson<sup>5</sup> found 30 cases of primary tumor, 7 of which were sarcoma. Ehrlenberg<sup>6</sup> collected 19 cases of sarcoma of the heart. Link<sup>7</sup> reported 91 cases, of which only 23 were of value from a clinical standpoint, and 13 specifically demonstrable as sarcoma. Up to 1877 Bodenheimer collected 7 cases of sarcoma of the heart, which had been found by Ely<sup>8</sup> in fifty years of the literature. Leyden and Fraenkel reported 2 cases, which were included in Beirhenson's summary. Of these 9 cases the round cell predominated in 8, the other being of giant cell type. Bernheim in 1890, reported a sarcomatous polyp of the right heart. Heektoen<sup>9</sup> reported a case of primary sarcoma of epicardium, and Brvant reported a similar lesion in the heart of a dog.

In 1896 Leroux and Mesely reported a primary sarcoma of the heart. Raw and Lambert reported<sup>10</sup> Raws' case, a spindle-celled sarcoma of the right auricle, Lambert<sup>11</sup> a primary angiosarcoma of heart and pericardium, described by Redtenbacher.<sup>12</sup> Azzuni, 1907, added another case of heart tumor. Baldwin<sup>13</sup> reported one case of primary large spindle celled sarcoma of heart, making a total of 17, to which may be added our own case. 18 in all. Except for a case reported by Armstrong and Monckeberg,<sup>14</sup> which was a lymph-angio-endothelioma, all cases were sarcomatous in origin.

A Matias,<sup>15</sup> April, 1927, reports from the literature 26 cases of sarcoma of the heart, 13 of which originated in the right auricle, mostly in the auricular septum. 1 case in both auricles, 2 in the left auricle, 3 in the right ventricle, 2 in the left ventricle, 1 in the endocardium of the aortic valve, 2 in the ventricular septum, 1 in the endocardium of the pulmonary valve, and 1 case the origin of which could not be determined because of the diffuse involvement of the entire heart. Peilstein<sup>16</sup> reports a case of cardiac sarcoma, originating in the subpericardial areolar tissue of a male forty-three years old. Adams states that cardiac neoplasm is rare due to activity, efficiency, nourishment and innervation.

Heart tumors most commonly encountered are fibromas and myxomas. Next in incidence are sarcoma. Joel<sup>17</sup> reports a teratoma. There also have been reported rhabdomyomas, lipomas, angiomas, lymphangiomas. Rhabdomyomas are usually congenital and in about 50 per cent of cases are associated with a tuberous sclerosis of the brain.

Of 2,942 necropsies at Johns Hopkins Hospital, only 10 heart tumors were found, all of which were secondary except 1 which was a rhabdomyoma, the remaining 9 being sarcoma 1, lymphosarcoma 1, epithelioma 2, endothelioma 1, and 4 carcinomas (Bryant<sup>18</sup>). From 1903 to 1907, 6,655 necropsies performed at the University of Berlin Pathological Institute revealed 15 secondary carcinomas and 4 secondary sarcomas of the heart (Karrenstein<sup>19</sup>). In the past

decade, in over 9,000 cases brought to autopsy at Kings County Hospital Morgue, the case herein described is the only one of primary sarcoma of the heart.

Thorel reports that in over 3,000 autopsies at Nuremberg, no cardiac neoplasm was seen.<sup>20</sup> The records at Bellevue Hospital<sup>21</sup> in over 7,000 cases show no cardiac primary neoplasm.

#### SUMMARY

This instance of primary sarcoma of the heart is reported in a case which unfortunately furnishes but little clinical history. The tumor is a small round-celled sarcoma, originating in the right auricle, which because of the apparent normal health of the deceased in almost fifteen years of his life, must have been insidious in onset, but after that a rapidly growing fatal neoplasm.

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# THE EFFECTS OF ADRENALECTOMY ON THE CARDIAC OUTPUT AND BLOOD PRESSURE\*

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THE alterations in the cardiac output and blood pressure following a number of procedures that result in acute circulatory failure have been determined. Blalock<sup>1</sup> found that the removal of blood from dogs caused a marked decrease in the output of the heart before a significant decline in the mean arterial pressure occurred. Similar findings were obtained by Johnson and Blalock<sup>2</sup> in experiments in which shock was produced by trauma to an extremity, by trauma to the intestines and by burns. Following the subcutaneous injection of histamine, the alterations appeared in the reverse order, there being first a drop in the blood pressure followed later by a decline in the cardiac output. The effects of trauma to the central nervous system were studied by Blalock and Bradburn.<sup>3</sup> The findings in these experiments were inconstant but usually the cardiac output and blood pressure declined simultaneously. The effects of spinal anesthesia were determined by Birch and Harrison.<sup>4</sup> They state, "From the data it appears that in spinal anesthesia the initial change is, as would be expected, in arterial pressure, and that the venous return and output of the heart are affected secondarily. This sequence of events is just the opposite of that in hemorrhage."

The present experiments were performed in an endeavor to obtain information concerning the nature of the circulatory failure that develops following the removal of the adrenal glands.

## METHODS

Dogs were used in all experiments. The operations were performed in two stages under ether anesthesia through lumbar incisions. The right adrenal gland was removed first. Approximately two weeks following the first operation, the dog was given one-half grain of morphine and an hour later the cardiac output and blood pressure were determined. The left adrenal gland was then removed. The animals were kept warm and observed closely. Additional determinations of the cardiac output and blood pressure were performed after varying intervals of time. When the animals were not quiet, morphine was given prior to the studies.

The arterial pressure was measured by a mercury manometer, the cannula being inserted into the femoral artery. Valves were used for determining the maximum and minimum pressures. The cardiac output was determined according to the Fick principle. Arterial blood was withdrawn from the femoral artery and mixed venous blood was obtained by puncture of the right ventricle. The Van Slyke-Neill apparatus was employed in determining the oxygen con-

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tent The oxygen consumption was determined by the use of a Benedict spirometer. The blood that was removed for the studies was replaced by an equal amount.

### RESULTS

Nine experiments were performed. In seven of the nine experiments, a rather marked decline in the blood pressure preceded any significant alteration in the cardiac output. In the remaining two experiments both the blood pressure and cardiac output had declined at the time of the second determinations. In three experiments in which early alterations in the blood pressure were detected, there was first a definite decrease in the maximum and a slight increase in the minimum pressures. This was associated with an increase in the pulse rate. The behavior of the animals differed from those previously studied in which shock was produced in various ways, in that the present animals appeared to be in fairly good condition and lived for a considerable period of time after the blood pressure had declined to a rather low level.

The results of a representative experiment are given in Table I.

TABLE I  
THE EFFECTS OF ADRENALECTOMY ON THE CARDIAC OUTPUT AND BLOOD PRESSURE

TIME	WEIGHT KG.	ARTERIAL O <sub>2</sub> VOL. PER CENT	VENOUS O <sub>2</sub> VOL. PER CENT	A-V DIF. VOL. PER CENT	MAX. B.P. MM. HG.	MIN. B.P. MM. HG.	O <sub>2</sub> CON- SUMPTION PER MIN. CC.	CARDIAC OUTPUT PER MIN. CC.
Control studies	15	12.61	7.22	5.39	145	81	121.0	2245
27½ hours post op		11.68	6.99	4.69	99	61	114.6	2443
28½ hours post op		12.76	7.86	4.90	99	61	120.5	2459
33½ hours post op		14.18	6.74	7.44	82	64	109.5	1476
40 hours post op		13.52	5.98	7.54	79	45	101.1	1341
45½ hours post op					45	39		

Protocol. Oct. 7, 1931, right adrenal gland removed. Nov. 11, 1931, 11 A.M. given ½ grain of morphine. 12 noon cardiac output and blood pressure determined. 5 P.M. left adrenal gland removed ether anesthesia. Nov. 12, 1931, 7:45 P.M. given one half grain of morphine. 8:30 P.M. and 9:30 P.M. determinations. Nov. 13, 1931, 2:15 A.M. determinations. 9 A.M. given ¼ grain of morphine. 9:30 A.M. determinations. 2:30 P.M. blood pressure determined. 2:45 P.M. dog died.

### DISCUSSION

The removal of both of the adrenal glands of dogs is followed by alterations in the cardiac output and blood pressure that are similar to those produced by the subcutaneous injection of histamine and by spinal anesthesia and probably to those occurring in primary shock. Following adrenalectomy both the output of the heart and the arterial blood pressure may remain at the normal level for a considerable period of time. Rogoff and Dominquez<sup>5</sup> found that the blood pressure following the removal of both adrenals remains elevated until several days preceding death. Their animals had a longer survival period than ours. This is due in part to the fact that the animals in the present experiments were given morphine from time to time in order to have them quiet during the studies. Morphine usually caused vomiting and diminished the amounts of food and water that were taken. Burwell and Smith<sup>6</sup> found that the low blood pressure in a patient with Addison's disease was associated with an essentially normal cardiac output.



Concerning the early deaths following adrenalectomy, Rogoff and Stewart<sup>7</sup> state, "As stated, we think that when dogs die in two or three days or less something else than adrenal insufficiency has contributed to shorten life, especially the direct consequences of the surgical procedure." If this is true, the operative shock is associated with alterations in the cardiac output and blood pressure unlike those accompanying hemorrhage, trauma to the intestines, trauma to an extremity, and burns.

## SUMMARY

The effects on the cardiac output and blood pressure of dogs of the removal of the adrenal glands have been studied. The initial alteration consisted of a definite decline in the arterial pressure followed later by a decrease in the output of the heart.

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# LABORATORY METHODS

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## A NEW BLOOD PLASMA CHLORIDE METHOD\*

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MERCURIC halides are very slightly dissociated and, due to this fact, several attempts have been made to develop a mercurimetric method for the determination of chloride. Little success was met with until Votoček<sup>1</sup> introduced sodium nitroprusside as an indicator.

When a standard mercuric nitrate solution is added, the chloride ions are removed as soluble, non-ionized mercuric chloride. After the removal of the chloride ions, white mercuric nitroprusside, which is insoluble in acid solution, is formed, thus producing a turbidity which indicates the end point.

Kolthoff and Bak<sup>2</sup> showed that the sensitivity of the indicator is reduced in the presence of the slightly dissociated mercuric chloride and gave the titration corrections to be used with various concentrations of mercuric chloride in water solutions.

Recently Fiedt Kok<sup>3</sup> applied the method to the determination of chloride in blood and serum, and recommended the use of sulphosalicylic acid as a precipitant for the proteins. The Folin-Wu filtrate was rejected because the reagents used to prepare the filtrate appeared to contain chlorides. The contamination of the filtrate is not obtained when Meick's C P sodium tungstate is used. Peters and Van Slyke<sup>4</sup> give a method for the testing and purification of the reagent.

It appeared that the above procedure, if suitably modified, would have certain advantages in the clinical laboratory over the standard methods now in use.

### METHOD

A Folin-Wu filtrate of the blood or plasma is prepared (1 c c of blood or plasma, 7 c c of water, 1 c c of  $2/3 N H_2SO_4$  and 1 c c of 10 per cent sodium tungstate are thoroughly shaken together and filtered).

Five cubic centimeters of the filtrate are pipetted into a large test tube and 0.2 c c (3 drops) of fresh 5 per cent sodium nitroprusside solution are

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\*From the Laboratory of Physiological Chemistry, University of Minnesota.  
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added Mercuric nitrate<sup>2</sup> (1 c.c. is equivalent to 1 mg. NaCl) is added from a micro burette until a permanent turbidity is produced on the addition of one drop

(Titration value in c.c. - X)  $\times$  200 = mg. of NaCl per 100 c.c. plasma

The titration corrections (X) which Kolthoff and Bak determined for varying concentrations of HgCl<sub>2</sub> in water are slightly low for the Folin-Wu filtrate. Accordingly, these factors have been redetermined for the Folin-Wu filtrate and are recorded in Table I

TABLE I

5 C.C. OF FOLIN WU FILTRATE		10 C.C. OF FOLIN WU FILTRATE*	
TITRATION VALUE IN C.C.	TITRATION COR RECTION $\lambda$	TITRATION VALUE IN C.C.	TITRATION COR RECTION $\lambda$
1.5	0.07	3	0.14
2.0	0.08	4	0.16
2.5	0.09	5	0.18
3.0	0.10	6	0.20
3.5	0.11	7	0.22

\*Should 10 c.c. of the filtrate be titrated the formula becomes (titration value in c.c. -  $\lambda$ )  $\times$  100 = mg. NaCl per 100 c.c. of blood or plasma

#### DISCUSSION

A number of samples of blood and serum have been analyzed by this method and check analyses made by the Whitehorn method, which was modified to the extent of centrifuging out the AgCl before titrating to the end point. The average variation of the results of the two methods on the different samples was 7.2 mg. NaCl per 100 c.c. of blood or plasma, with no regularity as to which method gave the higher result.

Accurate results may be obtained on 5 c.c. of a filtrate prepared from 9 c.c. of 2½ per cent sulphosalicylic acid and 1 c.c. of blood or plasma if the mixture is thoroughly shaken before filtering. However, the end point is slightly less distinct than with the Folin-Wu filtrate, as there is a tendency for the sulphosalicylic acid solution to form small bubbles when shaken. If centrifugation is used to separate the precipitated proteins from the filtrate, excellent results are obtained when the Folin-Wu precipitation is used, but a slightly turbid solution is obtained with the sulphosalicylic acid. Based on this observation the chlorides in 0.2 c.c. of finger tip blood can be determined in the following manner:

One and eight-tenths cubic centimeters of tungstic acid solution (7 volumes of water, 1 of sodium tungstate, and 1 of 2/3 N H<sub>2</sub>SO<sub>4</sub>) are placed in a centrifuge tube and 0.2 c.c. of blood or plasma are added from a pipette. The pipette is rinsed by drawing the fluid in and out, and the tube is thoroughly shaken. After centrifugation 1.0 c.c. of the supernatant fluid is re-

<sup>2</sup>Dissolve 1.833 g. of red mercuric oxide in an excess of concentrated nitric acid and dilute the solution to one liter. The solution should be checked by titrating against a standard NaCl solution (see Kolthoff and Furman Volumetric Analysis Vol. II John Wiley & Sons New York 1929 p. 219).

moved and titrated in a small test tube, using 10 drop of nitroprusside solution (Titration value  $-0.02$ )  $\times 1000 = \text{mg NaCl per } 100 \text{ c.c. of blood}$  The authors recommend that plasma be used instead of whole blood for the chloride in blood varies with the plasma corpuscle ratio due to the fact that blood cells contain only about half as much chloride per volume as plasma

#### SUMMARY

A mercurimetric method is given for the determination of chlorides in blood and plasma. By check determinations it has been found to be as accurate as the standard silver methods now in use.

The titration is made directly upon the Folin-Wu filtrate and the end point is easier to observe than that obtained with the methods now in use.

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### NOTE ON THE DETERMINATION OF PROTEIN IN SERUM BY A DIRECT MICRO KJELDAHL METHOD\*

ROGER S. HUBBARD, PH.D., AND GRACE E. SLY, B.S. BUFFALO, N. Y.

IN A previous note<sup>1</sup> one of the authors described a modification of the micro kjeldahl method for determining proteins in blood serum. In the present communication a further slight modification is described which has been in use in this laboratory during the last eighteen months, and which has been found to be very convenient. The method is based upon the separation of albumin and globulin by the technique of Howe,<sup>2</sup> oxidation of the solutions containing protein with the acid oxidizing reagent of Folin and Wu<sup>3</sup> and direct nesslerization of ammonia in the presence of Rochelle salt. Under the conditions described there is no precipitation of mercury salts and the colorimetric reading can be made without difficulty.

The technique used is described below.

**Apparatus**—(1) Folin digestion tubes of pyrex glass, graduated at 35 c.c. and 50 c.c., (2) Pyrex glass tubing of the smallest bore obtainable cut in 6 to 10 mm lengths, (3) micro burner, (4) ring stand with an adjustable clamp.

**Reagents**—(1) Nine tenth per cent sodium chloride, (2) concentrated sodium sulphate, prepared as follows: dissolve 22.2 gm of anhydrous salt (Merek's Blue Label, acid free) in 75 to 90 c.c. of hot distilled water, pour into a flask graduated

\*From the Buffalo General Hospital.

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at 100 c c and place in an incubator at 37°, place some distilled water in the incubator, when the temperature of the incubator is reached by both liquids, dilute the sodium sulphate to the mark with the distilled water, the solution is supersaturated at room temperature, and must be kept in the incubator, (3) 10 per cent sodium tungstate solution, (4) twelfth normal sulphuric acid, (5) Folin-Wu acid digestion mixture, prepared by pouring into 100 c c of distilled water an equal volume of a mixture prepared as follows to 50 c c of a 5 per cent copper sulphate solution add 300 c c of 85 per cent phosphoric acid and mix, add 100 c c of concentrated sulphuric acid (free from the least trace of ammonia) mix, protect from ammonia fumes, (6) Rochelle salt, a 10 per cent solution, (7) nitrogen standard, prepared by dissolving 0.1414 gm of the purest ammonium sulphate and making up to the mark in 100 c c volumetric flask with distilled water, a convenient dilute standard is made by diluting 10 c c of this stock standard to 100 c c in a volumetric flask with distilled water, (8) Nessler's solution prepared according to the directions of Folin and Wu, as follows 150 gm of potassium iodide and 110 gm of iodine are placed in a 500 c c Florence flask, 100 c c of water and an excess (140 to 150 gm) of metallic mercury are added, the flask is then shaken continuously for seven to fifteen minutes until the color of the iodine has disappeared and is replaced by the greenish color of the double iodide. The solution, which becomes quite hot, must be cooled under the tap when the color begins to get pale and before the red has wholly disappeared. Then decant, wash the mercury and flask with a large volume of water and make the supernatant fluid and washings up to two liters. The final Nessler's solution is prepared from this double iodide and from 10 per cent sodium hydroxide, which should be standardized with an accuracy of at least 5 per cent and which must be free from carbonate. To ensure the latter, prepare a strong solution of sodium hydroxide containing 55 gm in each 100 c c and allow to stand for several days or weeks until the sodium carbonate has precipitated and settled, then decant and dilute to a strength of 10 per cent just before using. To prepare the final Nessler's solution mix 3500 c c of this 10 per cent sodium hydroxide, 750 c c of the double iodide solution described above, and 750 c c of distilled water. The alkalinity of this solution is important, 20 c c of normal hydrochloric acid should require between 11 and 11.5 c c of the finished Nessler's solution to give an endpoint with phenolphthalein. The solution is clear when first prepared, if a precipitate forms, the supernatant fluid may safely be decanted and used.

*Preparation of Solutions for Analysis*—For total protein dilute 0.5 c c of serum accurately to 25 c c with 0.9 per cent sodium chloride, take 0.5 c c and proceed with the digestion as described below. Determine albumin after removing globulin in the following manner treat 0.5 c c of the serum with 15 c c of the 22.2 per cent sodium sulphate solution for three to four hours. Filter in an incubator using a No. 50 Whatman filter or a paper of similar grade. To protect against loss by evaporation, the funnel should be placed directly in the mouth of the flask and the top of the funnel covered with a watch glass. If the filtrate is not perfectly clear return to the filter paper. Take 0.5 c c of the filtrate, which contains albumin but not globulin, and proceed with the digestion. For the nonprotein nitrogen add to 2 c c of the serum, 16 c c of twelfth normal sulphuric acid and 2 c c of 10 per cent sodium tungstate. Mix and filter. Take 5 c c of the filtrate and proceed with

the digestion (Should the blood urea nitrogen be above normal use less of the filtrate, i e, when the blood urea nitrogen is more than 100 mg per 100 c c take only 1 c c of the filtrate )

*Digestion*—Into Pyrex test tubes graduated at 35 and 50 c c, measure the different solutions prepared as described in the preceding paragraph. Add 1 c c of the digestion mixture and 1 piece of Pyrex tubing. Place the tube in an adjustable clamp so that the bottom of the tube is about one inch above a micro burner, and heat with a flame that just touches the tube until the water is expelled and white fumes appear. Cover the tube with a watch glass and heat until the brown color entirely vanishes. Remove flame, cool until white fumes have nearly subsided and tilt the tube to an almost horizontal position. With a curved medicine dropper, carefully place a few drops of distilled water at the mouth of the tube and let them run down the sides. When the water mixes with the digested material, some bumping occurs but nothing will be lost if the water is added cautiously. As soon as the bubbling ceases make up to a volume of 10 to 15 c c with distilled water. Cool to room temperature. For the standards take 3 c c and 5 c c of the dilute solution (equivalent respectively to 0.09 and 0.15 mg nitrogen) in Pyrex tubes like those used for the unknowns, and add 1 c c of digestion mixture to each. Add 1 c c of 10 per cent Rochelle salt to the standards and unknowns. Make all tubes up to 35 c c with distilled water, add 15 c c of Nessler's solution, mix thoroughly and compare each unknown with the standard nearer to it in color.

#### Calculations —

$$N \text{ P N} = \frac{S}{U} \times St \times \frac{1000}{V}$$

$$T \text{ P} = \left[ \left( \frac{S}{U} \times St \times \frac{100}{0.01} \right) - N \text{ P N} \right] \times \frac{6.25}{1000}$$

$$Alb = \left[ \left( \frac{S}{U} \times St \times \frac{200}{0.0323} \right) - N \text{ P N} \right] \times \frac{6.25}{1000}$$

$$Glob = T \text{ P} - Alb$$

Where S = reading of standard, U = reading of unknown, St = value of standard in mg nitrogen, T P = total protein, Alb = albumin, Glob = globulin. Total protein, albumin, and globulin are expressed as grams per 100 c c, nonprotein nitrogen as mg per 100 c c. V = volume of filtrate used.

When the oxidation technique was applied to solutions containing urea and glucose the results were entirely satisfactory. For standardizing the method, further results obtained by it on a series of ten sera were compared with results given by a macro kjeldahl procedure on 3 and 5 c c of the same specimens. In these controls the final determination was made by titration with standard acid after the ammonia had been distilled from a sulphuric acid digestion mixture. The averages of the values of the two series differed from each other by only 0.6 per cent. A comparison of results when the micro method was carried out upon  $\frac{1}{2}$  c c and 1 c c of the same filtrate was also made. Oxidation was more difficult when the larger amount was used, and agreement between determinations not as close as in the figures just cited. However the average difference in ten determinations was only 1.6 per cent and the authors think such discrepancies are not greater than should be expected.

when a colorimetric method of this sort is used. The method described has been in use in this laboratory for eighteen months, and between 150 and 160 determinations have been carried through in that time. It has apparently been satisfactory within the limits expected of a routine method of this type which involves the measurement of small amounts of serum and the use of the colorimeter. The method certainly possesses a marked technical advantage over the one previously described by one of us.

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## A RAPID METHOD FOR THE SIMULTANEOUS DETERMINATION OF CARBON DIOXIDE CAPACITY AND UREA NITROGEN CONTENT OF BLOOD\*

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IN 1914 Van Slyke and Cullen<sup>1</sup> and Van Slyke and Zacharias<sup>2</sup> showed that urea is converted quantitatively into ammonium carbonate by the action of urease. Pantos<sup>3</sup> and Mirkin<sup>4</sup> showed that it is possible to estimate exactly the carbon dioxide liberated from the converted ammonium carbonate upon treatment with an acid. In 1927 Van Slyke<sup>5</sup> described a method for the determination of urea in blood by gasometric measurement of the carbon dioxide liberated from the ammonium carbonate formed by the action of urease. However, that method requires a large expensive apparatus, an experienced technician, and many carefully prepared reagents. These factors inhibit its use by the average physician or by a technician in the smaller laboratory.

The method described in this paper is simple and rapid. It can best be done on a simple, inexpensive, portable apparatus described by one of the authors.<sup>6</sup> This apparatus can be obtained from the Emil Greiner Co., New York.

## REAGENTS USED

- (1) 1.13 per cent KH<sub>2</sub>PO<sub>4</sub> in 0.02N H<sub>2</sub>SO<sub>4</sub>\*\*
- (2) Urease Solution†
- (3) Caprylic Alcohol

\*From the Pathological Laboratories of the Jewish Hospital and Crown Heights Hospital. Received for publication June 16, 1932.

\*\*Phosphate Reagent. Take 11.3 gm. KH<sub>2</sub>PO<sub>4</sub> and dissolve in approximately 500 c.c. distilled water. Add 2.8 c.c. of 10N H<sub>2</sub>SO<sub>4</sub>. Make up to a liter with distilled water.

†Urease Solution. Make fresh for each determination. In a small test tube (4 by 3/8) dissolve contents of a No. 1 capsule of Urease Squibb in 1 c.c. of Phosphate reagent. Add 1 drop of Caprylic Alcohol. Shake well until it is finely emulsified.

## DRAWING OF BLOOD SAMPLES

Puncture a vein with an ordinary intravenous needle and allow about 5 cc of blood to flow into a tube containing a small pinch of potassium oxalate. Cork immediately. Invert the tube 2 or 3 times and let the plasma separate by gravity. Do not centrifuge. The analysis should be done as soon as cells have settled.

## PROCEDURE

Raise the apparatus to position "A" (Fig 1) by loosening the thumb-screw. Clean the apparatus by introducing 1 cc of phosphate reagent into the chamber. Lower and raise the leveling cup a few times and eject the solution by opening

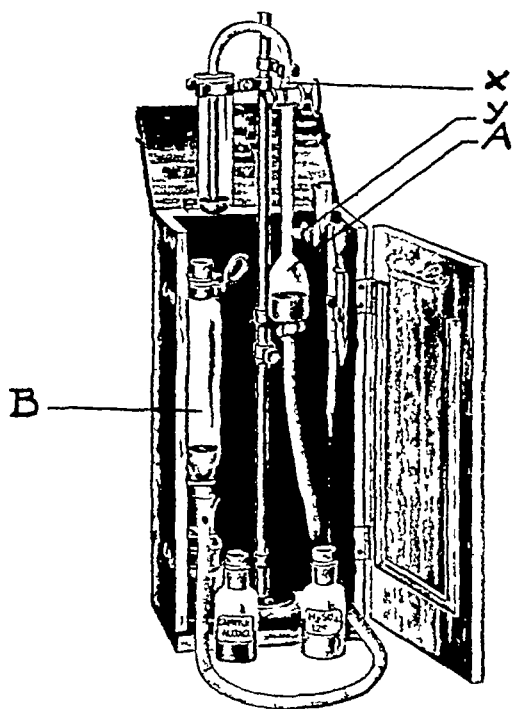


Fig 1

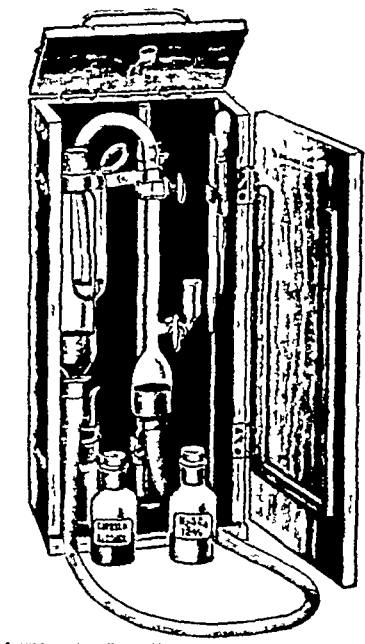


Fig 2

stopcock "X." Produce a vacuum by lowering the mercury leveling cup. Raise the leveling cup. If the apparatus is gas free, the mercury will strike the upper cock with a sharp click. If this click is not heard, allow the accumulated gas or fluid to escape by opening the upper cock "X," and repeat lowering and raising the mercury leveling cup until the click is heard. With the leveling cup in position "B" introduce 0.5 cc of serum or plasma into the side arm. Add one drop of caprylic alcohol on top of the serum or plasma. Rotate the stopcock slowly until most of the fluid in the side arm enters the chamber and displaces the mercury from above. No harm is done if the mercury column in the chamber is broken by the entrance of the serum or plasma.



Wash the cup with 0.5 cc of the phosphate reagent drawn up with the same pipette and allow it to enter the chamber, leaving just enough solution in the cup so that no air is possibly introduced. Seal the stopcock by running a drop of mercury into the capillary "Y." Lower and raise the mercury leveling cup a few times in order to wash down all serum or plasma adhering to the walls of the stem. Lower the leveling cup until the mercury drops to the 25 cc mark. Tip the box gently back and forth for one minute, thus agitating the solution in the chamber. This may also be done by tapping the sliding rod. The mercury in the leveling cup is now raised slowly so that it reaches the level of the mercury meniscus in the stem. Take  $R_1$  and note the amount of CO<sub>2</sub> in 100 cc of blood by using Table I. Now introduce all of the prepared urease solution into the cham-

TABLE I

$R_1$ OR $R_2$	VOL % CO EQUIV TO $R_1$ 2*	MG % UREA N EQUIV TO $R_2$ 3†	$R_1$ OR $R_2$	VOL % CO EQUIV TO $P_1$ 2*	MG % UREA N EQUIV TO $R_2$ 3†	$R_1$ OR $R_2$	VOL % CO EQUIV TO $R_1$ 2*	MG % UREA N EQUIV TO $R_2$ 3†
1	2*	3†	1	2*	3†	1	2*	3†
0 030		1 2	0 185	23 6	37 2	0 350	55 5	75 6
0 035		2 3	0 190	24 6	38 4	0 355	56 7	76 7
0 040		3 5	0 195	25 5	39 5	0 360	57 4	77 9
0 045		4 7	0 200	26 5	40 7	0 365	58 4	79 1
0 050		5 8	0 205	27 5	41 9	0 370	59 4	80 2
0 055		7 0	0 210	28 4	43 0	0 375	60 3	81 4
0 060		8 1	0 215	29 4	44 2	0 380	61 3	82 6
0 065		9 3	0 220	30 3	45 4	0 385	62 3	83 7
0 070		10 5	0 225	31 3	46 5	0 390	63 2	84 9
0 075		11 6	0 230	32 3	47 7	0 395	64 2	86 0
0 080		12 8	0 235	33 2	48 8	0 400	65 2	87 2
0 085		14 0	0 240	34 2	50 0	0 405	66 1	88 4
0 090		15 1	0 245	35 2	51 2	0 410	67 1	89 5
0 095		16 3	0 250	36 1	52 3	0 415	68 1	90 7
0 100		17 4	0 255	37 1	53 5	0 420	69 0	91 8
0 105		18 6	0 260	38 1	54 7	0 425	70 0	93 0
0 110	9 1	19 8	0 265	39 1	55 8	0 430	71 0	94 2
0 115	10 1	20 9	0 270	40 0	57 0	0 435	71 9	95 3
0 120	11 0	22 1	0 275	41 0	58 1	0 440	72 9	96 5
0 125	12 0	23 3	0 280	42 0	59 3	0 445	73 9	97 7
0 130	13 0	24 4	0 285	42 9	60 5	0 450	74 8	98 8
0 135	13 9	25 6	0 290	43 9	61 6	0 455	75 8	100 0
0 140	14 9	26 7	0 295	44 9	62 8	0 460	76 8	101 2
0 145	15 9	28 0	0 300	45 8	64 0	0 465	77 8	102 3
0 150	16 8	29 1	0 305	46 8	65 1	0 470	78 7	103 5
0 155	17 8	30 2	0 310	47 7	66 3	0 475	79 1	104 6
0 160	18 8	31 4	0 315	48 7	67 4	0 480	80 5	105 8
0 165	19 7	32 6	0 320	49 7	68 6	0 485	81 6	107 0
0 170	20 7	33 7	0 325	50 7	69 8	0 490	82 6	108 1
0 175	21 7	34 9	0 330	51 6	70 9	0 495	83 6	109 3
0 180	22 6	36 0	0 335	52 6	72 1	0 500	84 5	110 5
			0 340	53 6	73 3			
			0 345	54 5	74 4			

\*The values in Column 2 are the same as those given by Van Slyke except that they have been modified to correspond to  $\frac{1}{2}$  the quantity of plasma or serum used and have been corrected for CO<sub>2</sub> contained in the Phosphate reagent and caprylic alcohol.

†The values in Column 3 have been calculated from the following formula At 20° C

$$\text{Mg \% Urea N} = R_2 - P_1 - 0.025$$

0 0043

The value 0.025 is a correction for the constant volume of CO<sub>2</sub> liberated from blank determinations of the PO<sub>4</sub> urease and caprylic alcohol reagents.

ber, leaving just enough in the cup so that no air is possibly introduced. Seal the stopcock by running a drop of mercury into capillary "Y". Lower and raise the mercury leveling cup a few times in order to bring the urease solution in contact with the plasma or serum which wets the walls of the stem. Lower the leveling cup until the mercury in the chamber is at the 25 c.c. mark. Shake for two minutes. Then raise the leveling cup to position "B". Introduce 1 c.c. of the phosphate reagent into the chamber, leaving just enough solution in the cup so that no air is possibly introduced into the chamber. Seal capillary "Y" with a drop of mercury.

Lower leveling cup so that mercury in the chamber reaches the 25 c.c. mark. Shake for one minute. Raise mercury in leveling cup slowly until it reaches the level of mercury in the chamber. Take  $R_2$ . The difference between  $R_1$  and  $R_2$  gives  $R_3$ . Note amount of urea nitrogen in blood corresponding to  $R_3$  from Table I.

#### DISCUSSION OF TABLE I

Both the  $\text{CO}_2$  combining power and the Urea Nitrogen content of blood can be read directly from this table. For example

$$\text{If } R_1 = 0.345$$

$$\text{and } R_2 = 0.435$$

$$\text{then } R_3 = 0.090$$

The value in Column 2 corresponding to  $R_1$  or 0.345 in Column 1 is 54.5 volumes per cent of  $\text{CO}_2$ . The value in Column 3 corresponding to  $R_3$  or 0.090 in Column 1 is 15.1 mg. per cent of urea nitrogen.

TABLE II

COMPARISON OF  $\text{CO}_2$  DETERMINATIONS WITH THE SIMPLIFIED APPARATUS AND THE VAN SLIKE APPARATUS

BLOOD NO	SIMPLIFIED	VAN SLIKE	VOLUME PER CENT DIFFERENCE
1	26.5	24.2	2.3
2	40.0	38.5	1.5
3	56.7	54.8	1.9
4	40.0	39.5	0.5
5	34.2	31.9	2.3

#### DISCUSSION OF TABLE II

Table II shows slight differences between the two methods. Considering the original Van Slyke method as the standard, the simplified method is accurate with 1.7 volumes per cent  $\text{CO}_2$ .

TABLE III

COMPARISON OF UREA NITROGEN DETERMINATIONS DONE BY THE SIMPLIFIED METHOD AND BY THE VAN SLIKE CULLEN TITRATION METHOD

BLOOD NO	SIMPLIFIED	VAN SLIKE CULLEN	MG PER CENT DIFFERENCE
1	29.1	28.5	0.6
2	25.6	26.5	0.9
3	67.4	65.0	2.4
4	14.0	15.1	1.1
5	42.6	43.9	1.3

## DISCUSSION OF TABLE III

Table III shows a number of analyses done by the simplified method and the Van Slyke-Cullen Titration method. The difference in the results vary from 0.6 to 2.4 mg with a maximum error of 7 per cent.

TABLE IV  
REPRODUCIBILITY OF THE SIMPLIFIED METHOD

BLOOD NO	VOLUMES % CO <sub>2</sub>	AVERAGE DEVIATION	MG % UREA N	AVERAGE DEVIATION
1	45.8	0.4	11.6	0.0
	45.8		11.6	
	44.9		11.6	
2	40.0	0.3	50.0	1.0
	39.1		50.0	
	39.1		52.3	

## DISCUSSION OF TABLE IV

Table IV shows extremely consistent results with the simplified method.

## DISCUSSION

The simplified apparatus is particularly suited for the CO<sub>2</sub> and urea nitrogen determinations because of the wide range over which it yields accurate results. The simplified CO<sub>2</sub>-Urea method has several advantages over the ammonia estimation method for urea. It dispenses with apparatus required for the aeration or distillation of the ammonia. Apparatus for titration and colorimetric reading are not necessary. Exact standard solutions for titration or for colorimetric comparison in nesslerization are eliminated. The result is a diminution in sources and likelihood of error, and a gain in rapidity.

The phosphate reagent is of sufficient acidity to liberate all of the CO<sub>2</sub> (see Table II) and yet weak enough to produce optimum buffer conditions so that the action of urease is not inhibited. The urease itself being alkaline neutralizes the slight excess of phosphate reagent present and brings the P<sub>H</sub> up to the proper level. Under these conditions the action of the urease is almost instantaneous even with uremic blood.

If the simplified method is used for the analyses of fluids other than plasma or serum, then the P<sub>H</sub> of the sample used must be carefully adjusted.

## SUMMARY

1. A simple rapid method is described for the simultaneous determination of CO<sub>2</sub> and urea nitrogen on 0.5 c.c. of blood.
2. The determination may be done on plasma or serum.
3. The accuracy for CO<sub>2</sub> determinations is within 3 volumes per cent.
4. The accuracy for urea nitrogen determinations is within 6 per cent.

The results obtained with 0.5 c.c. samples are equal in accuracy to those obtained with the usual 3 c.c. samples by the ammonia titration method. Hence only 1/6 the amount of blood is required by the simplified method.

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## THE ROSE BENGAL TEST OF HEPATIC FUNCTION\*

### A SPECTROSCOPIC METHOD

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DYE elimination tests of hepatic function have proved their usefulness and are at present a part of the armamentarium of the internist. The reliability of such tests depends to a great extent upon the nature of the dye used and on the method by which its elimination through the liver is determined.

Rose Bengal† (di-iodo-tetra-chloro-fluorescein) is very suitable for this purpose because it is excreted exclusively by the liver. The technic of the test as described by Epstein, Delprat and Kerr<sup>1</sup> has the important advantage of eliminating artificial standards by using as a standard for comparison the concentration of rose bengal in the patient's blood two minutes after the injection of the dye. In this way the significance of all individual peculiarities of weight, blood volume, etc., with the consequent possible errors are eliminated. In addition all adults can receive the same amount of rose bengal, making calculation of dosage unnecessary.

There remain two sources of error inherent in the colorimetric reading of samples which are overcome by the spectroscopic method to be described. The first of these is the deeper yellow color of the eight minute and sixteen minute samples due to lowering of the concentration of the dye and the consequently greater thickness of the layer of plasma necessary to match the two minute specimen. This is especially noticeable in normal and borderline cases and is more pronounced in the sixteen minute sample where it often amounts to matching intensities of different colors.

Attempts to overcome this difficulty by precipitating the blood proteins with acetone were unsuccessful because a sufficiently large and inconstant

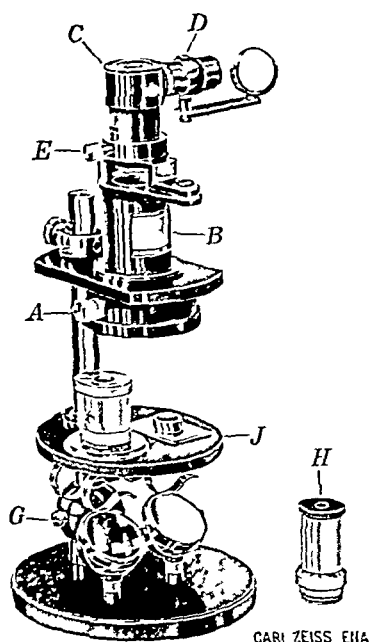
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†This dye may be obtained in ampule form from the firm of Hynson Westcott and Dunning Baltimore Maryland

portion of rose bengal is carried down in the precipitate to distort the results of the test. The second potential source of error in the colorimetric method is the presence in the blood of increased amounts of pigment or colloidal substances, such as bilirubin in jaundice, hemoglobin due to hemolysis, and lipoids in postprandial or diabetic states.

In order to avoid these difficulties, Snapper and Spoor<sup>2</sup> proposed the use of the spectroscope for quantitative determinations of the dye per 100 cc of plasma. Their method involves the combined use of a Hellige colorimeter and a spectroscope, the readings being made on the colorimeter when the absorption band of rose bengal is just visible in the spectroscope. While a step in the right direction, this method depends on the use of artificial



CARL ZEISS JENA

Fig 1.—Comparison spectroscope with absorption vessel of variable depth of fluid

standards and for accuracy requires identical conditions in regard to intensity of illumination and aperture of the spectroscope.

The method proposed here is based on the use of a comparison spectroscope (such as seen in Figs 1 and 2) and combines the advantages of the colorimetric and spectroscopic methods.

**Technic**—Ten cc of a 1 per cent solution of rose bengal in triple distilled water is injected into a vein of one arm. Two minutes, eight minutes, and sixteen minutes after the injection, 5 cc of blood is collected from a vein of the other arm into a syringe and transferred to test tubes. The syringe and test tubes are previously rinsed with a solution of potassium oxalate to prevent coagulation. The blood samples are centrifuged until the erythrocytes separate from the plasma. The latter is pipetted off and di-

luted with twice its volume of distilled water. From here on the procedure depends on the type of spectroscope used. If a comparison spectroscope similar to the one shown in Fig 1 is available, the two minute and one of the other samples of diluted plasma are placed in the cups of the instrument. The absorption band of rose bengal appears in the green part of the spectrum ( $555\text{ }\mu\mu$ ) and is of greater width and intensity in the two minute specimen.

By manipulation of the screw the thickness of the plasma layer in the two minute sample is reduced until the absorption bands are equal in width and intensity. Then the figures on the scale are read and the percentage of the dye in the second sample easily calculated assuming the two minute specimen to contain 100 per cent of rose bengal. The same procedure is repeated using the two minute and the remaining samples.

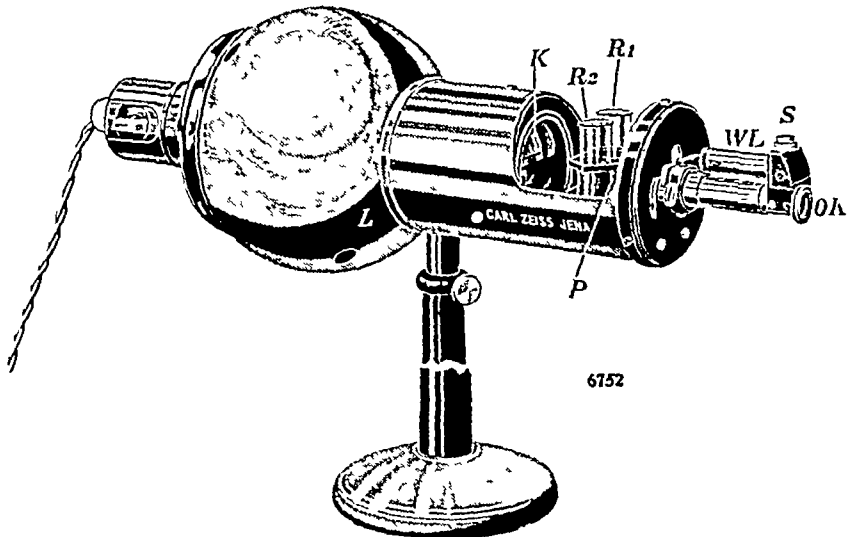


Fig 2—Hand spectroscope with test tube condenser

If a simpler hand spectroscope shown in Fig 2 is used the amount of diluted plasma in the test tubes must be measured. It is best to begin with the two minute (A) and the sixteen minute (C) samples, and by gradually adding measured amounts of water to Tube A, effect a match of the two absorption bands. The concentration of the dye in Tube C is calculated according to the following formula  $X = \frac{A \times 100}{A + A_1}$ , where A is the amount of plasma in the first specimen, and  $A_1$  the amount of water added to Tube A. Following this the eight minute (B) sample is diluted in the same manner to match the sixteen minute (C) sample, and the concentration of rose bengal in Tube B is obtained from the following equation  $Y = \frac{X (B + B_1)}{B}$ , where B is the amount of plasma in the second specimen and  $B_1$  the amount of water added to Tube B.

With either type of spectroscope, no precautions need to be observed regarding the intensity of illumination or aperture of the instrument

The figures thus obtained indicate the amount of rose bengal still present in the circulating blood eight and sixteen minutes after the injection of the dye

In order to ascertain the practical value of the spectroscopic method of reading the rose bengal liver function test, both the spectroscopic\* and the colorimetric methods were carried out on identical blood samples in 100 instances

## RESULTS

1 *Normal Cases*—In 37 patients with normal elimination of rose bengal, the average of spectroscopic readings for both samples was 68 per cent higher than that of the corresponding readings with the colorimeter. The significance of this difference is that the upper limit of normal which has been accepted for the colorimetric method as 50 per cent of the standard at the end of eight minutes, and 30 per cent at the end of sixteen minutes, must be changed to 55 per cent and 35 per cent respectively if the spectroscopic method is used. The explanation of this difference probably is that the yellowish color of the plasma obscures the dye to a greater extent in the last two specimens

2 *Cases With Hepatic Damage*—In 42 individuals with abnormal retention of rose bengal, the average of all spectroscopic readings was 56 per cent higher than that of the colorimetric readings, thus confirming the quantitative differences observed in 37 normal cases

3 *Interfering Substances in the Plasma*—a *Jaundice* 16 rose bengal tests were performed on 13 patients with clinical jaundice having an icterus index from 15 to 105. Of these it was impossible to carry out the colorimetric determination on 13 eight minute samples and on 14 sixteen minute samples. On the other hand the presence of bilirubin in the plasma did not hamper the spectroscopic determinations of rose bengal in any of the samples

As a crucial test the plasma of the eight and sixteen minute specimens from a case of jaundice with an icterus index of 81 were diluted with the icteric plasma of the same patient instead of water and still the spectroscopic readings could be carried out with ease and were equal to those obtained in the usual manner. In a patient with hemolytic jaundice having an icterus index of 30 the colorimetric readings were modified by the icterus to show abnormal retention of rose bengal whereas the spectroscopic determinations revealed normal excretion of the dye

b *Hemolysis* On four occasions considerable hemolysis was visible in the centrifuged plasma. In all cases the colorimetric reading was either impossible or much higher than the spectroscopic one. In the spectroscope the presence of hemoglobin is manifested by two absorption bands ( $540\text{ }\mu\mu$  and  $580\text{ }\mu\mu$ ), one on each side of the rose bengal band. All four specimens could be read satisfactorily. However, when very marked artificial hemolysis is

\*The hand spectroscopic shown in Fig. 2 was used for this work. Since then the instrument shown in Fig. 1 has been employed in 150 cases with excellent results

produced the hemoglobin bands encroach on the rose bengal band and prevent even the spectroscopic determination

c Lipemia In one case of marked cholesteremia (1075 mg of cholesterol per 100 c.c. of blood), the colorimetric reading of the eight minute sample was abnormally high whereas the sixteen minute sample could not be matched satisfactorily. There were no difficulties in making determinations with the spectroscope on either sample. Hence it is no longer necessary with this method to delay the rose bengal test for four hours after a meal.

*Summary*—A spectroscopic method of reading the rose bengal liver function test is described. This method retains all advantages of the colorimetric method and renders the test more reliable. In addition it permits the test to be carried out in spite of jaundice, moderate hemolysis and lipemia.

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### SUPERSATURATION OF ANTIGENIC BEEF-HEART EXTRACTS WITH CHOLESTEROL AND ITS EFFECT ON THE SENSITIVITY AND SPECIFICITY OF THE COMPLEMENT FIXATION REACTION\*

B. S. LEVIN, PH. D., CHICAGO, ILL.

PORGES, Neubauer and their collaborators<sup>1</sup> showed that as the percentage of cholesterol in relation to lecithol in solution increases, the precipitation of this complex by human serum, and especially by positive syphilitic serum, increases abruptly to a maximum. Further increase in the cholesterol content leads to a gradual reduction in the precipitation. In a previous publication I stated that the same held true with regard to the complement fixation reaction.<sup>2</sup> The following experimental series were designed for the purpose of investigating this phase of the complement fixation reaction.

Dry beef-heart powder was rendered free from ether-soluble substances. It was extracted with 95 per cent grain alcohol for five days at room temperature. This was labeled Extract II. The same beef-heart powder was again extracted with hot grain alcohol, and the material labeled Extract I. Two hundred and fifty cubic centimeters of Extract I were evaporated to dryness and the residue redissolved in 200 c.c. of Extract II. This was labeled Extract III.

To portions of these extracts cholesterol was added as indicated in Table I. Antigen titrations were carried out as per Kolmer.<sup>3</sup> Final values were obtained by averaging the dilution at which a very faint tint of red appeared (denoted in the tables as V. F. T. R.) and the dilutions at which there appeared a barely observable increase in the red tint. The results are recorded in Tables I, II and III.

\*From the Clinical Laboratory of the Public Health Institute  
Received for publication August 30, 1932



TABLE I  
EXTRACT I

% CHOLESTEROL	V F T R. IN DILUTION	SIT INCREASE, IN DILUTION	AVERAGE DILUTION
0.00	1 1200	1 1800	1 1500
0.25	1 3600	1 3800	1 3700
0.50	1 5000	1 5400	1 5200
1.00	1 4800	1 5200	1 5000
2.00	1 4200	1 4600	1 4400

TABLE II  
EXTRACT II

% CHOLESTEROL	V F T R. IN DILUTION	SIT INCREASE, IN DILUTION	AVERAGE DILUTION
0.00	1 2800	1 4800	1 3800
0.60	1 4400	1 5200	1 4800
0.80	1 4800	1 5600	1 5200
1.00	1 5600	1 7200	1 6400
1.50	1 7200	1 9600	1 8400
2.00	1 8000	1 10800	1 9400
2.50	1 8200	1 10800	1 9700

TABLE III  
EXTRACT III

% CHOLESTEROL	V F T R. IN DILUTION	SIT INCREASE, IN DILUTION	AVERAGE DILUTION
0.40	1 5200	1 6200	1 5700
0.60	1 7000	1 8000	1 7500
0.80	1 8400	1 9000	1 8700
1.00	1 9100	1 11200	1 10150
1.50	1 10400	1 12400	1 11400
2.00	1 10800	1 13500	1 12150
2.50	1 10000	1 12000	1 11000
3.00	1 10000	1 12000	1 11000
3.50	1 8000	1 10000	1 9000

The tables show that in complement fixation as in antigen precipitation the increase in the antigenic properties of the reagent with the increase in the percentage of cholesterol reaches a maximum which may or may not be the optimum. Beyond that maximum the antigenic power of the reagent becomes inversely proportional to the cholesterol content.

In the instance of Extract I the maximum was attained with 0.5 per cent cholesterol, in the case of Extract II, with 2.5 per cent cholesterol, and in the case of Extract III, with 2 per cent cholesterol. Evidently the percentage of cholesterol required to bring the beef-heart extract to its maximum reactivity depends upon the lipid concentration of the extract, beyond a certain lipid concentration the percentage of cholesterol required to manifest the maximum reactivity of the antigenic extract also begins to decline. This is in accord with the general principles of colloidal reactions, and follows the so called "colloidal type curve" discussed in another paper.

The data recorded in Tables I, II, and III likewise show that a reagent having any desired antigenic titer can be obtained for the complement fixation reaction by

manipulating the lipid concentration of the alcoholic extract and its ratio to the cholesterol content Eagle<sup>9</sup> prepared an alcoholic extract containing 0.6 per cent cholesterol and 0.6 per cent sitosterol (corn germ sterol). This reagent gave an antigenic titer as high as 1-10000 dilution. Using an especially strong positive serum and Extract II (normal alcoholic beef-heart extract) fortified with 1.25 per cent cholesterol, I obtained a reagent which gave an antigenic titer as high as 1-10200 dilution. It is not necessary, therefore, to complicate the reaction by the use of more than one type of sterol to obtain a reagent possessing an antigenic titer as high as 1-10000 or higher.

All serologic laboratory reactions based on alcoholic beef-heart extract, fortified with sterol, as the antigenic reagent, are basically immunologic reactions. However, they are specific for syphilis only when kept at an experimentally established level of sensitivity. The sensitivity of a fortified beef-heart extract in complement fixation is determined by its lipid content and the percentage of sterol added, while the proper balance between sensitivity and diagnostic specificity is attained by establishing the proper ratio between the beef-heart lipid and the fortifying sterol. This ratio varies with each extract. The chemistry of the lipid in particular and of the fixation reaction in general is still insufficiently known. A direct and scientific method for the standardization of the antigenic reagent is therefore yet to be developed. At the present time the correspondence between the laboratory results and the clinical findings of a large number of cases remains the only rational guide in judging the suitability of an antigenic reagent for routine laboratory purposes, and since the reaction per se is nonspecific, it cannot be assumed a priori in every case that the reagent possessing the highest titer must prove the most sensitive and at the same time the more specific.

TABLE IV  
EXTRACT I

SPEC NO	COLM	FAHN	PERCENTAGE OF CHOLESTEROL AND DILUTION			
			10% CHOL 1:200	0.5% CHOL 1:550	1.0% CHOL 1:480	2.0% CHOL 1:440
1	00	00	0	0	2	Tr
2	00	00	0	0	1	0
3	00	00	0	2	3	2
4	00	00	0	0	0	4
5	00	00	0	0	0	4
6	00	00	0	0	3	0
7	00	00	0	1	4	4
8	00	00	0	1	3	1
9	00	00	P M*	4	4	4
10	00	00	0	0	1	0
11	32	0 Tr	0	0	0	0
12	00	00	0	2	3	3
13	00	00	0	0	2	0
14	00	00	0	Tr	2	0
15	00	00	0	0	2	0

\*P-M = Plus-Minus Numerals 1 2 3 4 indicate degrees of complement fixation Tr = trace  
0 = negative for fixation

TABLE V  
FRACTION II

SPEC NO	KOLMER	KAHN	PERCENTAGE OF CHOLESTEFOL AND DILUTION					
			0.4% 1:350	0.6% 1:450	0.8% 1:520	1.0% 1:610	1.5% 1:840	2.0% 1:940
1	00	00	Tr	Tr	1	1	3	3
2	22	00	3	3	3	3	3	4
3	00	Tr Tr	0	0	0	0	1	4
4	00	00	0	0	0	0	1	3
5	4 Tr	00	4	4	4	4	4	4
6	00	00	Tr	2	3	3	4	4
7	00	00	Tr	2	3	3	4	4
8	00	11	0	0	0	0	3	4
9	00	00	0	0	0	0	1	1
10	00	00	0	0	0	0	1	3
11	00	00	0	0	0	0	0	1
12	00	00	0	0	0	0	0	1
13	00	00	0	0	0	0	0	1
14	00	00	0	0	0	0	1	1

TABLE VI

SP NO	KOLM	KAHN	PERCENTAGE OF CHOLESTEFOL AND DILUTION									
			0.4%	0.6%	0.8%	1.0%	1.5%	2.0%	2.5%	3.0%	3.5%	
			1 670	1 750	1 870	1 1000	1 1150	1 1200	1 1100	1 1100	1 900	
1	00	00	0	0	0	0	1	1	1	Tr	0	
2	0 Tr	01	1	3	3	4	4	4	4	4	4	
3	00	00	0	0	0	0	Tr	Tr	2	1	Tr	
4	00	00	0	0	0	0	1	1	1	1	0	
5	22	44	0	2	3	3	4	4	4	4	4	
6	00	00	0	0	Tr	1	3	3	3	3	3	
7	00	00	0	0	0	0	0	0	0	1	0	
8	00	00	0	0	0	0	0	0	0	1	0	
9	00	00	0	0	0	0	0	0	0	1	0	
10	00	00	0	0	0	0	Tr	0	1	1	Tr	
11	00	00	0	0	0	0	0	0	0	1	0	
12	00	00	0	0	2	3	4	2	2	1	1	
13	00	00	0	0	0	0	1	Tr	Tr	1	Tr	
14	00	00	0	0	Tr	1	1	2	4	4	3	
15	00	00	0	0	0	0	0	0	1	0	0	
16	00	00	0	0	Tr	Tr	1	2	3	4	4	
17	00	00	0	0	0	Tr	1	2	4	4	3	
18	00	00	0	1	3	4	4	4	4	4	4	
19	00	00	0	0	0	0	0	0	0	1	0	
20	00	00	0	0	0	0	0	0	0	1	0	
21	00	00	0	0	1	1	2	3	3	3	3	
22	00	00	0	0	0	0	0	4	0	0	0	
23	00	00	0	0	0	0	Tr	4	1	Tr	0	
24	00	00	0	0	0	0	0	3	0	0	0	
25	00	00	0	0	0	0	0	4	0	0	0	
26	00	12	0	1	3	3	4	4	4	4	4	
27	00	00	0	0	0	0	0	4	0	0	0	
28	00	00	0	0	0	0	0	4	0	0	0	
29	11	00	0	3	3	4	4	4	4	4	4	
30	00	00	0	0	3	3	4	4	4	4	4	

The following experiments support these views

Fifty-five blood-serum specimens were tested by the antigens prepared from Extract I, 72 specimens were tested by the antigens prepared from Extracts II and III. The dilutions of the antigens used represented ten units of the corresponding titers. Of the 55 specimens tested by the antigens prepared from Extract I, 31 were negative and 9 were positive by the experimental procedure and by the standard Kolmer<sup>4</sup> and by the two-tube Kahn test,<sup>7</sup> which were performed in all cases for control purposes. The results of the remaining 15 specimens were recorded in Table IV. Forty-seven of the specimens tested by the antigens prepared from Extract II were negative and 10 were positive by the experimental and standard routine procedures. The results obtained with the remaining 14 specimens are recorded in Table V. Thirty-three of the specimens tested by the antigens prepared from Extract III were negative and 9 were positive by all the tests employed. The results of the remaining 30 specimens are recorded in Table VI.

A brief summary of the histories of the cases, the results of which are recorded in Table VI, follows

CASE 5—Syphilis latent (1938), treated. Clinical examination, spinal fluid, and routine serologic tests negative

CASE 6—Had syphilitic infection twenty five years ago. Reported to the Institute for a check up examination. Routine serologic tests negative

CASE 9—Kahn test occasionally gives a plus minus reaction, while the Kolmer test is consistently negative. Patient is under observation for syphilis

CASE 18—Neurosyphilis. Routine serologic tests are consistently negative

CASE 21—Neurosyphilis. Routine serologic tests at first were weakly positive. Following treatment they became persistently negative

CASE 26—Cardiovascular syphilis. Routine serologic tests are inconsistent and weak, with the Kolmer predominantly negative

CASE 27—Syphilis secondary, recurrent. Routine serologic tests, at first positive, became consistently negative following treatment

CASE 29—General paresis. Routine serologic tests inconstant. On some occasions all routine and special tests gave negative results

CASE 30—Central nervous system syphilis, asymptomatic, late. Kolmer test towards end of treatment became consistently negative, and the Kahn test giving inconstant weakly positive results. Finally all tests became consistently negative

Of the remaining 29 cases none had any clinical or routine serologic findings suspicious of syphilis. With a few exceptions these cases were under treatment for gonococcus infection

#### DISCUSSION

By increasing the lipid and cholesterol content of the antigen, its titer and sensitivity can be raised so that reactions will result from serum specimens of nearly all patients having had syphilis. The following considerations tend to indicate that such a procedure can hardly be approved at the present

1. As stated in one of the preceding paragraphs, the complement fixation reaction is specific only within clinically established limits. If its sensitivity is raised much above the level of the presently recognized routine tests, the incidence of non-specific reactions will increase considerably

2 The transition from primary to the early and late secondary stages of syphilis is associated with definite changes in the intensity of the serologic reaction<sup>8</sup>

3 The reduction in the degree of positivity of blood tests is regarded as an encouraging index of efficient therapy

4 Repeated negative serologic results are important criteria of cure when clinical findings also indicate that the progress of the disease has been arrested

If through the introduction of supersensitive antigens all serums from syphilitic individuals, regardless of the amount of treatment received, should be made to yield strongly positive results, then new criteria for judging the progress of the infection and of the effectiveness and sufficiency of treatment would have to be evolved

The Kolmer complement fixation and the Kahn antigen precipitation tests have been in my experience thoroughly trustworthy for routine purposes. The technical details are well defined in each, the level of reactivity of each strikes a practical medium proved by years of extensive experience, they are supplementary to each other. Nevertheless I am not of the opinion that the combination of the two tests offers an all-sufficient and infallible system of serologic study. In isolated instances special tests should be employed. However, when evaluating the results of tests of high sensitivity the clinician must not forget that the incidence of nonspecific positives increases in direct proportion to the increase in the sensitivity of the test.

Special tests should be based on as near the same principles and general technical steps as are the adopted standard routine tests. The introduction of new fortifiers, such as corn germ sterol leads the busy clinician to new confusion. Cholesterol alone accomplishes the same degree of sensitizing for the fixation reaction as the mixture of the two sterols. It appears logical to me that for the sake of simplicity and technical uniformity cholesterol continue to be used as the exclusive fortifying sterol in the preparation of the antigenic reagent for use in laboratory diagnosis of syphilis by complement fixation.

#### SUMMARY

The increase in the antigenic properties of alcoholic beef-heart extract with the increase in the cholesterol content reaches a maximum for complement fixation. This maximum depends upon the lipid concentration of the alcoholic solution. Beyond the maximum fortification the antigenic power of the reagent becomes inversely proportional to the cholesterol added.

Generally, the higher the titer of the fortified beef-heart extract, the greater is its sensitivity. When the full antigenic dose of the fortified reagent is used its sensitivity can be manipulated, on the basis of the principles discussed above, so that strongly positive reactions are obtained with serum specimens from nearly all individuals who have had syphilis, regardless of the age of the infection and of the intensity of treatment received. Such a high level of sensitivity results in the appearance of strongly positive reactions, basically of an immunologic character, but considered "false positives" from the viewpoint of the syphilologist.

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## DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KUDLFFE, M.D., ABSTRACT EDITOR

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**TISSUE** A Device for Mounting Anatomical Preparations, Schoenburn, H. W., and Hickman, C. P. *Science* 77: 287, 1933

A plate is made by melting hard paraffin and pouring it into a form (as a box top, etc.) where it hardens to form a plate from one fourth to one half inches in thickness. During the process of melting, enough lampblack is added to give the mixture a deep black color, which is, of course, an advantage where a dark background is desired. To prevent curling of plates after immersion in preservative, two glass rods are embedded parallel to each other, one on each side of the plate, just before the process of hardening is begun. These rods may be of various diameters, but those from 5 to 8 mm in diameter are quite satisfactory. The length of the rods will depend upon the length of the plate as they are placed lengthwise within the plate. Before the rods are dropped in the melted paraffin a loop or two of heavy twine is wrapped around each end of each rod so that the rods will be suspended near the middle of the plate after it hardens. Care must be taken to smear the inside of the box form with glycerine so that the hardened plate can be removed easily from the form.

The addition of some beeswax and resin to the paraffin lampblack mixture will increase to a considerable extent the rigidity of the plate, although these constituents are not absolutely necessary.

Before the plate has hardened, and is still plastic, the anatomical specimen is pressed down slightly into the soft plate to hold it in position and is fastened there with thread looped around the parts of the specimen, passed through holes, and tied at the back of the plate. Necessary labels are now attached.

When completed, the preparation is immersed in a preservative in a museum jar. Such preparations will last for an indefinite time.

**ENDOSPORES**, Simplified Method of Staining, Schaeffer, A. B., and Fulton, M. D. *Science* 77: 194, 1933

Stains —

1 Five per cent aqueous malachite green. Dissolve, allow to stand thirty minutes, filter.

2 0.5% aqueous safranin.

Method —

1 Prepare films and fix as usual.

2 Flood with malachite green and heat to steaming three or four times within one minute.

3 Wash under tap water thirty seconds.

4 Apply safranin stain thirty seconds.

5 Wash, blot dry, and examine.

Spores green, cells red.

**TISSUE** Improving the Staining Action of Iron Hematoxylin, Hance, R. T. *Science* 77: 287, 1933

*Stock Solution* — Hematoxylin 10 per cent in 95 per cent alcohol.

*Staining Solution* — Five c.c. of stock solution to 100 c.c. of distilled water. Add as much sodium bicarbonate as can be lifted on the point of a scalpel. The neutralized stain gives excellent differentiation.

# **THYROID DISEASE** Blood Cholesterol In, Hursthal, L M Arch Int Med 51 22, 1933

The blood cholesterol during fasting was determined as a routine in 505 cases of thyroid disease, along with the pulse, the weight, and the basal metabolic rate

The lowest average values for blood cholesterol are found in patients in or near thyroid crises

Auricular fibrillation in toxic goiter is associated with the next lowest average level of cholesterol

The average cholesterol value in all types of exophthalmic goiter is lower than in toxic adenomatous goiter

Recurrent hyperthyroidism is associated with cholesterol values that are almost as low as those in exophthalmic goiter

The average cholesterol value in nontoxic goiter is normal, although the scatter is wider than expected Age appears to raise the blood cholesterol in these cases

Chronic thyroiditis is associated with higher average values than in any other thyroid disease except myxedema

The level of the blood cholesterol and the basal metabolic rate bear a reciprocal relationship when judged by average values

# **ARTHRITIS, Rheumatoid, Cecil, R L J A M A 100 1220, 1933**

The modern method of approach to the problem of chronic arthritis is based on the following considerations

The majority of cases of chronic arthritis fall into one or the other of two great groups Rheumatoid arthritis is a clearly defined clinical entity which in most cases can be accurately differentiated from osteoarthritis and from the so called specific forms of infectious arthritis Rheumatoid arthritis has its own pathology and usually runs a characteristic clinical course Hypertrophic arthritis, on the other hand, appears to be a degenerative, senescent process

Evidence is rapidly accumulating that rheumatoid arthritis (like rheumatic fever) is a chronic infection This evidence comes from pathologic, bacteriologic, serologic, and clinical sources Allergy probably exerts an influence on the disease, but no more than in other chronic infections

In rheumatoid arthritis a primary focus of infection is the portal of entry for the exciting agent In respect to its pathogenesis, the disease is analogous to gonococcal arthritis, which it often resembles closely The foci and the infectious agents are different, the mechanism of infection the same

By employing new bacteriologic methods, a number of investigators have succeeded in cultivating streptococci from the blood and joints of patients with rheumatoid arthritis While these observations have not been confirmed in some laboratories, the presence of specific streptococcal agglutinins in the serum of rheumatoid patients would seem to refute the theory that these microorganisms are contaminations Furthermore, the ease with which an experimental arthritis can be produced in rabbits by intravenous injection of these streptococci lends strong support to the proposition that rheumatoid arthritis is a streptococcal infection

The specific streptococcal agglutinins which are found in the serums of a high percentage of patients with rheumatoid arthritis, are of considerable value in differentiating rheumatoid arthritis from other forms of chronic arthritis The sedimentation rate is useful in separating rheumatoid arthritis and the other infectious forms from noninfectious ailments of the joints

By making use of the agglutination and sedimentation tests and the Schilling count, the physician is now in a position not only to make a more accurate classification of arthritic patients but also, by repeating the tests from time to time, to gauge the activity of the disease and the efficacy of treatment



The rational treatment of rheumatoid arthritis embraces removal of foci of infection, emphasis on rest, and a general building up program, consisting of carefully regulated diet, vitamins, iron and arsenic, and adequate elimination through the intestine, bladder, and skin. Streptococcus vaccine, administered intravenously, is of considerable value in many cases, though its modus operandi is not yet understood. Physical therapy, if carefully supervised, is useful in stimulating the local and general circulation. In advanced cases, orthopedic surgery may solve an otherwise baffling problem.

**TISSUE** A New Paraffin Embedding Mixture, Hance, R. T. Science 77 353, 1933

Difficulty in obtaining a paraffin of satisfactory consistency in which to embed and section biologic material is the development of the following formula

A stock solution of crude rubber in paraffin is first made. Crude rubber is available in thin sheets, either smoked or unsmoked. Both kinds work equally well. The sheets of crude rubber are chopped up with a scissors and dropped in melted Paraffin or any similar common paraffin. The paraffin should be smoking hot and the mixture should be stirred occasionally. Three to four hours are required to completely melt the rubber. About 20 gm. of rubber can be dissolved in 100 gm. of paraffin.

**Embedding Mixture**

Paraffin	100 gm
Rubber paraffin mixture	45 gm
Beeswax	1 gm

Filter through paper (paper towels serve this purpose excellently)

This mixture is pale yellow in color, does not crystallize readily, and is of a waxy consistency that sections unusually well.

**TISSUE** Microtechnical Demonstration of Insoluble Lime Salts in Tissues, Gomari, G. Am J Path 9 253, 1933

- 1 Cut or saw thin blocks of fresh tissue 1 to 2 mm thick
  - 2 Fix in 80 to 96 per cent alcohol, or boil in one of the solutions mentioned. After this wash blocks in distilled water for three to four hours.
  - 3 Impregnate in 15 per cent silver nitrate solution for six to ten days. Change silver solution once or twice.
  - 4 Wash for three to four days in distilled water changed daily four to five times, until the last washing water decanted does not show the slightest turbidity when mixed with hydrochloric acid.
  - 5 Reduce in a 5 per cent solution of sodium hypophosphite. Before use add 4 to 5 drops of a 0.1 normal sodium hydroxide solution to each 100 cc of reducer. Keep blocks in reducer for four to eight days.
  - 6 Wash in running water for three to four hours.
  - 7 Fix in a 3 to 5 per cent solution of sodium thiosulphite for two days.
  - 8 Wash in running water for at least twenty four hours.
  - 9 Decalcify in a 6 to 8 per cent solution of sulphosalicylic acid.
  - 10 Wash, embed, and so on.
- Steps 3 to 7 inclusive are to be performed in the dark.

**RENAL FUNCTION**, In Arterial Hypertension, Ellis, L. B., and Weiss, S. J. A. M. A. 100 875, 1933

In 24 cases of arterial hypertension without clinical signs of cardiac or renal failure, and in eight cases of glomerulonephritis, three types of tests of renal function were used: the usual urinalysis, urea and creatinine clearance tests, and concentration dilution tests.

In 10 of the cases of hypertension the urea clearance test gave normal results, in 9 there was a slight reduction, and in 5 it was markedly lowered.

Creatinine clearance tests, in 18 of the same cases, was normal in 13 instances, slightly reduced in 2, and markedly reduced in 3. In general, the outcome of the urea and creatinine tests tended to give parallel results.

In 22 cases in which the concentration test was performed in the patients with hypertension, the maximum specific gravity of the urine was above 1.025 in six instances, 10 times it fell between 1.020 and 1.025, and in the remaining 6 cases it fell below 1.020.

In only 2 cases did the concentration test show a definite lowering without a marked reduction in the urea or creatinine clearance tests.

For practical purposes, in most cases a carefully conducted concentration test is as sensitive an index of renal impairment as the urea and creatinine clearance tests.

Marked albuminuria or hematuria was uncommon in hypertension unless great limitation of function was detectable by other tests.

The results of this study have been analyzed in accordance with the filtration reabsorption theory of renal physiology and have been correlated as far as possible with the histologic changes in the kidney, known to occur in hypertension.

There was a trend for the degree of impairment of renal reserve to parallel the height of the arterial blood pressure, particularly the diastolic pressure. There was no correlation to be made between the degree of renal damage and the age of the patients, the symptoms or the known duration of the disease.

**SPORES, A Simplified Method of Staining, Shaeffer, A. B., and Fulton, M. Science 77 194, 1933**

Films of bacteria, made in the usual manner, are fixed by flaming three times. Flood with 5 per cent aqueous malachite green and heat to steaming 3 or 4 times within one half minute. Wash off the excess stain under the tap for about one half minute. Apply a 0.5 per cent aqueous safranin solution for one half minute. Wash, blot, dry, and examine. Spores are stained green, cells red. There is no blending of the colors.

**MALIGNANCY, A Quantitative Modification of the Bendien Reaction in Serodiagnosis of, Lowe, E. C. Brit. M. J. 3376 407, 1933**

With the technic described below, which is stated to have received Dr. Bendien's approval, the author reports 95 per cent diagnostic accuracy if the clinical condition is not taken into account and 98 per cent if it is.

His conclusions are as follows from a study of 600 tests:

The primary tube reaction alone is shown to be insufficient for the diagnosis of malignancy. The triple test must be employed, and will then differentiate malignant from nonmalignant conditions in at least 95 per cent of cases.

The clinical malignancy and the degree of positiveness shown by the test closely correspond.

Postoperative cases of cancer, or those having radium, x-ray, or other forms of treatment, may be "followed up" by means of this test, and clinical information may thereby be obtained regarding prognosis and treatment.

Cases are referred to in which satisfactory clinical improvement and apparent cure is associated with the continued presence of a normal or nonmalignant serum reaction.

In some cases the serum reaction has foretold a recurrence months before clinical observations have confirmed it.

Among normal and clinical nonmalignant conditions a certain percentage of abnormal reactions occur, suggesting the possibility of recognizing in such cases a precancerous tendency.

The test is delicate and reliable, more satisfactory as a laboratory technic than any other at present available, and should be of value as a clinical aid to diagnosis and prognosis of malignancy, especially in early cases.

The technic follows

*The Reagents*—The first eight mixtures of N/10 acetic acid and N/10 ortho sodium vanadate are prepared according to Bendien's detailed description

*The Serum*—Ten cc of blood is required, obtained from the patient in a fasting condition. The serum should not be hemolyzed, after standing it is centrifuged clear of cells. Fine's suggestion of using only 0.1 cc of serum in each reaction tube economizes serum.

The tubes must be standardized, 75 mm in length and 7 to 8 mm internal diameter. Gross variations cause considerable alterations in reaction.

Delivery of the small quantities of serum and reagents employed demands absolute accuracy.

For the modified test now to be described each serum is divided into three portions and marked accordingly. Approximately 1 cc is required for each part.

"A" is the portion to be used unheated. It is carefully diluted with an equal quantity of distilled water, well mixed, and 0.2 cc of the mixture is delivered into each of eight tubes, the respective reagents are added, Reagent 1 to the first tube, Reagent 2 to the second, etc., the mixtures well shaken, and put aside to settle for sixteen to twenty-four hours. "B" is the portion to be inactivated by heating in a water bath for half an hour at 56°. "C" is the portion in which the lipid protein complex is to be dissociated by ether. For this purpose at least half as much ether is added as there is serum, the whole being thoroughly shaken in a well corked tube and set aside to separate out. This will occur as a layer above the cleared serum.

The following day the "A" (unheated serum) tubes are examined. The reactions vary from nil in which the contents of the tube are quite clear, to a complete precipitation of all protein contents of the serum, which is shown by a heavy deposit lying below a water clear, supernatant fluid. Between these two extremes every variety of turbidity and less or more precipitation may be found. Two rows of tubes are now placed in the rack behind the "A" series. Into the first of these rows 0.2 cc of diluted "B" (heated) serum is placed, and into the second row 0.2 cc of diluted "C" (ether treated) serum, which has been carefully pipetted from below the ether layer and then diluted. To each tube 1 cc of the respective reagents is added, all being shaken and allowed to stand, as in the case of the "A" series. From each serum a series of the "A," "B" and "C" series may differ markedly in certain cases, according to the amount of thermolabile substance inactivated by heating and to the dissociation effect produced by ether on the serum contents.

#### ESTIMATION OF DEPOSIT

The visual readings of the deposits, although they show a move to the right of varying amount in the "B" (heated serum) and a move to the left, most marked in malignancy, in the "C" (ether treated serum) reaction, are not sufficiently accurate for quantitative estimation, and the following method is therefore employed. The tubes are centrifugalized at about 1,000 revolutions per minute and the supernatant fluid carefully removed without disturbing the deposit. The deposits are dissolved by adding, to each, 2 cc of N/100 NaOH, and the resulting solutions vary in concentration according to the amount of precipitate dissolved in each. By means of the Lowe interferometer (1910), as made by Zeiss, these concentrations are read in units, using a 5 mm observation chamber, and by experimental estimation it is found each unit corresponds to 0.025 mg. A reading, therefore, of 40 units equals 1 mg of precipitate, and this amount obtained from 0.1 cc of serum equals a 1 per cent precipitation. Experimentally this amount of deposit is found to be that which usually appears visually as the "first precipitation," and it was therefore arbitrarily taken that a quantitative estimation of 40 units (or nearest above) should be regarded as the "first precipitation" and the reaction classified by the reagent number in which this amount of deposit is first found.

## INTERPRETATION OF FINDINGS

The tube of the "A" series in any case which gives the first reaction having been determined, the unit measure thus obtained is taken as the basal figure for the computation of that serum, and the corresponding "B" and "C" deposits are similarly estimated, with the result that from these values in interferometer units a fraction may be worked out in which the numerator is calculated by subtracting the unit value of "A" from "C" and the denominator by subtracting "B" from "A"

Reagent	6	A	B	C	6	A	B	C
Interferometer		40	20	45		59	57	109
units of deposit								
Fraction $\frac{C - A}{A - B} =$			5/20				50/2	
			= Nonmalignant				= Malignant	

In cases where the thermolabile effect is marked and the increase of precipitation after ether treatment is slight (as is found in normal or nonmalignant inflammatory conditions) the fraction is less than unity. Conversely, when the thermolabile effect is slight or absent, and the precipitation after ether treatment increased or high (as is found in malignancy), the resulting fraction is equally consistently greater than unity, and this differentiation holds good irrespective of whether the first "A" reaction occurs in the lower or in the higher numbered tubes. Normal serums, as a rule, show a first precipitation with Reagent 6. Nonmalignant infective and malignant conditions may show a first reaction in any of the series according to the severity of the pathologic condition.

# TUBERCULOSIS Tuberculin and the Schilling Differential Blood Count in the Diagnosis and Classification of Tuberculosis, Bredeck, J. F. *Am. Rev. Tuberc.* 27: 377, 1933

From a study of the two reactions in a series of 305 cases, the following conclusions are drawn:

1. The interpretation of the clinical importance of the subcutaneous tuberculin test can best be judged by the Schilling blood differential count.
2. The Schilling count is a more accurate measure of induced focal reactions than are fever, general tuberculin reactions, physical signs, or x-ray.
3. A classification of the various stages of activity as well as those of healing can best be made by the Schilling count together with the subcutaneous tuberculin test.
4. The subcutaneous tuberculin test together with the Schilling count is the most delicate and most accurate method in the diagnosis of early manifest and induced focal activity.

On the basis of his findings, the validity of the author's classification (see below) originally proposed in 1929 is reaffirmed.

**"Active Tuberculosis"**—A patient who has a degenerative neutrophilic shift to the left with the Schilling blood differential, due to tuberculous infection, with a further shift to the left of the neutrophilic leukocytes or a drop in the lymphocytes following 10 mg. or less of Old Tuberculin.

**"Potentially Active Tuberculosis"**—A patient who has a normal or a degenerative neutrophilic shift in the Schilling differential, of doubtful origin, and who has an increased degenerative neutrophilic shift or a drop in lymphocytes following 10 mg. of Old Tuberculin. This is associated with a general tuberculin reaction with fever.

**"Apparently Healed"**—A patient who has a normal or a degenerative neutrophilic shift in the Schilling blood differential, of doubtful origin, and who has an increased degenerative neutrophilic shift or a drop in lymphocytes following 10 mg. or less of Old Tuberculin, not associated with a general tuberculin reaction with fever.

**"Healed Tuberculosis"**—A patient who has a normal or degenerative neutrophilic shift to the left in the Schilling blood differential of doubtful origin, and who fails to get any change in the Schilling blood differential following 10 mg. of Old Tuberculin.

**MELANURIA, Blackberg, S N, and Wanger, J O J A M A 100 334, 1933**

The following test for melanin is proposed as more reliable than those in common use

1 A twenty four hour specimen of urine is evaporated to one fourth of the original volume

2 One gram of potassium persulphate is added for each hundred cubic centimeters of the concentrated urine

3 At the end of two hours, in equal volume of absolute methyl alcohol is added The precipitated melanin is allowed to settle

4 The precipitate is filtered off and washed with water till the washings are colorless, then washed with methyl alcohol, to remove any soluble pigments remaining Finally, it is washed with ether If the test is positive there remains on the filter paper a brownish black precipitate, which can be dissolved off with alkali, most conveniently with 5 per cent sodium hydroxide Acidification of the alkaline solution causes a reprecipitation of the melanin

**ACETONE, Detection of in Urine, Leti, F Chum Ind Agr biol 8 419, 1932**

Place 1 gm of a mixture of 4 gm of sodium nitroprusside and 10 gm of dry sodium hydroxide in a porcelain dish and add 1 to 2 drops of urine In the presence of acetone a red lilac color at once changing to brown appears In the absence of acetone the red lilac color first appearing turns canary yellow

The reaction is said to respond to as little as 0.025 per cent of acetone

**BLOOD, Occult, Demonstration of, Mediriger, P Deutsche Ztschr ges Gericht Med 20 74, 1932**

The following method is stated to be both delicate and reliable

**Reagents**

A "Leucomalachite green"	1 gram
Glacial acetic acid	100 c c
Distilled water	150 c c
B 1 per cent hydrogen peroxide	

**Method**—One drop of a mixture of 8 parts of A to 2 of B is added to the material to be examined In the presence of blood a dark green color appears within ten seconds turning to dark bluish green in one minute

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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### A Textbook of Pathology

THOSE who are familiar with MacCallum's Pathology, and their name is legion, will welcome this fifth edition which has been extensively and thoroughly revised in accordance with the changes which have occurred, and the newer views now accepted in this field.

The plan and character of the book remain unchanged and based upon the conception that 'all pathologic disturbances are the result of some form of injury or of the immediate or remote reactions of the body to injury.'

The sections on tuberculosis, syphilis, endocarditis and disturbances of the organs of internal secretion have been revised in accordance with the newer conceptions of these subjects. New sections have been added, notably those upon infections of uncertain character, diseases due to injury of the organs or internal secretions, diseases due to specific dietary insufficiencies, disturbances of lipid metabolism, and diseases of undetermined origin affecting bones. Hodgkin's disease is now discussed in a separate chapter.

While not intended as a book of reference the volume will undoubtedly, as before, be widely read and frequently consulted for its clear and logical presentation of the subject.

There is a full index and the typography and excellent reproduction of the equally excellent illustrations are deserving of the highest commendation.

The book can be recommended without reserve not only to the student and physician but also to the worker in this field desirous of refreshing his memory, either of the basic principles, or the newer conceptions in the field of pathology.

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### Dosage Tables of Roentgen Therapy†

THIS book should be particularly useful in clinics devoted to roentgen therapy. About one half of the book consists of dosage tables, the first portion describing the methods by which the tables were compiled and the various factors entering into their construction.

The book is not to be taken as presenting, as it were, prescriptions for roentgen therapy, but is concerned mainly with a consideration and estimation of physical quantities which are presented in a concise and understandable manner. The depth dose through a portal of definite size, the effective dose, and the skin dose can all be readily determined, although some practice in the use of the tables will be necessary to eliminate probable sources of error.

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### Therapeutic Uses of Infra-Red Rays‡

ONE would gather from a perusal of this book that 90 per cent of the aches and pains of mankind can be relieved if not cured by infra red rays. There may be some conditions which infra red rays would cure but heat would not alleviate, but they are not to be found mentioned in this book.

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\*A Textbook of Pathology. By W. G. MacCallum, M.D. Professor of Pathology and Bacteriology, The Johns Hopkins University, Baltimore, Md. Fifth Edition. Thoroughly Revised. 1212 pages with 652 illustrations. Philadelphia and London 1932. W. B. Saunders Co.

†Dosage Tables of Roentgen Therapy. By Frederick Voltz, Head of The Radiological Department, University Clinic for Women, Munich. Cloth 120 pages. Oxford University Press.

‡Therapeutic Uses of Infra-Red Rays. By W. Ansdall Troup, M.C. M.B. Ch.B. Cloth 57 pages 16 plates. The Active Press Ltd.

The author details the cure of nearly every medical condition except the acute infections and the metabolic diseases, among them abscesses and bronchitis, carbuncles and cataracts, earache and epididymitis, frostbite and leukopenia, interior polyomyelitis, tooth ache and writer's cramp

The illustrations show various types of apparatus for the application of infra red rays, apparently in every instance stock cuts bearing the manufacturer's name

The opening paragraphs of the chapter on technique follow "It is very important—indeed imperative—to have a spacious, bright, and well ventilated room for treatment. I would suggest that the walls should if possible be distempered in primrose or pale blue and the curtains preferably blue"

This volume can hardly be recommended as a dispassionate or scientific presentation of the place of infra red rays in the treatment of disease

### Sex and Internal Secretions

AS STATED in the preface, this is not—nor was it intended to be—"a popular book on sex" Its purpose is "to survey the most important recent researches in the problems of sex, especially those concerned with internal secretions, in order that the concepts already established by experimental evidence may be clearly stated and readily available"

To this end, under the editorship of Dr Allen, a group of investigators whose work has established them in their respective fields, have contributed in this volume a comprehensive and authoritative presentation of the many notable discoveries and factual contributions which have accumulated during the past decade in the realms of general biology, physiology, and psychology of sex

It would be impossible within the limits of an ordinary book review to outline, even cursorily the volume and extent of the data discussed within this book Some idea of its extent may be had from the table of contents which follows the general biologic introduction.

Interrelation of Genic and Endocrine Factors in Sex (C H Danforth), Genetics of Sex in *Drosophila* (C B Bridges), Embryological Foundations of Sex in Vertebrates (B H Wilker), Sex Derivations, Inversions, and Parabiosis (E Witschi), Metabolism and Sex (O Riddle), Biology of the Testis (C R Moore), Biochemistry and Assay of Testis Hormones (F C Koch), Ovarian Follicular Hormone, Theelin, Animal Reactions (E Allen), Biochemistry of the Follicular Hormone, Theelin (E A Daisy), Physiology of the Corpus Luteum (F L Hisaw), The Mammary Glands (C W Turner), Plumage Tests in Birds (Domm, Juhn, and Gustavson), Ovulation, Transport, and Viability of Ova and Sperm in Female Genital Tract (C G Harman), Effect on the Reproductive System of Ablation and Implantation of the Anterior Hypophysis (P E Smith), Effects of Extracts of Anterior Pituitary and Similar Active Principles of Blood and Urine (E T Engle), Anterior Pituitary Changes Referable to the Reproductive Hormones and the Influence of the Thyroid and Adrenals on Genital Function (Severingham, Engle and Smith), Sexual Drive (C P Stone), Endocrine Disorders of Sex Function in Man (J P Pratt)

An extensive index of authors and of subjects is appended

To those interested in the problems of sex, those engaged in investigation, and those casting about for promising problems of investigation this volume should prove invaluable

The format is excellent and conforms to the motto of this house "Sans Toche"

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## EDITORIAL

### The Serum Bilirubin Test

FEW laboratory tests have commanded so much attention as the so-called van den Bergh test for bilirubin. "So-called" is used advisedly, because the test, first published by van den Bergh and Snapper in 1913, was modified by van den Bergh and Muller three years later, and it was not until 1918 that van den Bergh published alone and then did not add materially to the technique. Indeed the test itself may be looked on as an application of one devised by Ehrlich years before in which he showed that when diazonium salts were added to bilirubin dissolved in chloroform or alcohol a red color was produced when the solution was neutral, or a bluish color when the solution was acid. Proscher suggested the clinical use of the test a few years later. It would appear that McNee, the first to publish on the subject in English, was chiefly responsible for the term "van den Bergh test," for his paper contained the following



"He (van den Bergh) virtually rediscovered the color reaction given by bilirubin with the diazo reagent of Ehrlich. An attempt is evident in recent German papers to claim the test now applied to the serum in jaundice under the name of Ehrlich-Prosser reaction. There seems no doubt, however, that since van den Bergh first applied the reaction to the examination of blood serum, and has made such important original observations and deductions from its use, the test as applied to the study of jaundice should with fairness and right be known as the 'van den Bergh test'."

In spite of this statement the test represents clearly the work of at least four men. How much better and fairer to all concerned it would have been to have called the test the "diazo bilirubin test." For few tests, has so large a number of modifications been offered as for this one, since van den Bergh and his coworkers announced it, nor can one name a test, as useful and yet one around which so many errors have gathered.

It would not be profitable to mention the numerous errors in bibliographic references to the test nor the many errors made in ascribing certain statements to various authors who did not make such statements. However, it is very much worth while to record certain fundamental errors, since these have a practical bearing on the interpretation of the test itself.

As a setting for the discussion, it is desirable to give briefly the outstanding events in the history of the test after Ehrlich's contribution of 1883. In 1913 van den Bergh and Snapper published the tests as applied to blood serum, and in spite of van den Bergh's later statement that the test was simply an "estimation" and not a "quantitative determination" of bilirubin, they published the method first under the subtitle of "Eine quantitative Bestimmung des Bilirubin im Blutserum," the details set forth clearly pointed to quantitative considerations although as the matter eventually worked out the test certainly warranted only an "estimation." This paper concerned itself with the amount of bilirubin in different sera, not only of man but of other animals. The amounts were expressed in terms of concentration, and these in turn were allocated to ten classes. In performing the test the investigators added alcohol to the serum, centrifuged, withdrew an aliquot part of the supernatant liquid, and then added the Ehrlich reagent. They mentioned the volume of the protein precipitate and the contraction of the total volume due to the retraction of the mixture of alcohol and serum. These two items were taken into account in making the computations, as is clearly shown in the example cited in the paper.

In 1916 van den Bergh and Muller called attention to the fact that two kinds of reactions in serum could be shown to exist according to whether the red color developed before or after the addition of alcohol, one reaction they referred to as direct and the other as indirect. They began the development of the idea that the direct reaction occurred in serum from patients with obstructive (mechanical) jaundice and the indirect reaction occurred in serum from patients with hemolytic jaundice the latter also being the reaction of normal serum.

Van den Bergh, in his monograph of 1918, developed these ideas further and advanced two new procedures. In the first place he introduced an ether solution of iron rhodanate as a standard, and added 0.5 cc of alcohol after the first alcoholic precipitation and before adding the Ehrlich reagent. He also modified the calculation so as to ignore the volume of the precipitate, considering the serum as being diluted five times. He reported quantities under the term "Bilirubinwert." He placed his normal values as 1:400,000 to 1:250,000, or 0.5 to 0.8. In 1921 van den Bergh used the term 'unit' and defined it as being the equivalent of 1:200,000 dilution of bilirubin. Since 1:200,000 really means 0.5 mg per cent, one unit means 0.5 mg per 100 cc, although reporting on the basis of milligram values did not become popular until much later. In this paper van den Bergh gave as normal values 0.2 to 0.6 units, or 1:1,000,000 to 1:400,000\*. Apparently therefore McKee is not responsible for the translation of "Bilirubinwert" to "bilirubin unit" as stated by Hunter, and the confusion caused by such a term as "unit" if such there be, must rest jointly on Botzian (1920), Rosenthal (1920), van den Bergh (1921), and Thannhauser and Andersen (1921). The last authors used the German term "Bilirubinmenge" in relation to the van den Bergh reaction to mean a value of 1:200,000, although Rosenthal, in 1920, also used the word. It was used, moreover, by Lepehne in 1921 and probably originated with Botzian, a pupil of Rosenthal. Hunter's objection to the term is not taken on altogether sound grounds.

Van den Bergh also described the delayed direct reaction as appearing after several minutes, and thought that the bilirubin which would produce a direct reaction had passed through the polygonal cells of the liver and had been taken out by the blood stream instead of being excreted through the hepatic ducts. The bilirubin giving an indirect reaction he thought to be bound with serum albumin.

Thannhauser and Andersen, believing that some of the bilirubin was precipitated in the albuminous precipitate, modified the technique. They added the Ehrlich reagent first, then, after coupling had taken place (several minutes) they added alcohol and saturated solution of ammonium sulphate. They ignored the rather large colorless precipitate and heavy solution of ammonium sulphate. Their diazo reagent was five times the strength of the original reagent.

The matter of standards for comparison has received considerable attention. McNee and Keefer (1925), on the authority of van den Bergh and Muller introduced a standard of cobalt sulphate, Rhamy and Adams, a solution of potassium permanganate, Hall added sulphuric acid to the cobalt sulphate, and White added hydrochloric acid to the same salt. Nichols and Jackson used cobalt chloride and hydrochloric acid. All these standards have been used in various colorimeters and in transverse comparison tubes.

Although numerous modifications of the test have appeared, it should be noted that from time to time authors have called attention to the fact that temperature, length of time elapsing after withdrawal of blood, and the  $P_H$ , all have important bearing on the results, and that the natural evaporation of

\*There is obviously an error in these figures since 1:400,000 is 0.5 units.

both alcohol and ether in the ethereal standards will greatly alter the values derived

The mode of determining the type of reaction is also a question Feigl and Queener early discovered that in some sera, after an initial development of color some further color developed, and the reaction was called biphasic. Unfortunately, Lepelme's modification in the form of a ring test has been generally overlooked, although it appears to be the most accurate method of determining the type of reaction present Elton, a decade later, redescribed the ring test as an innovation Various rules have been laid down concerning the time to wait for the appearance of the color, little agreement exists, although the general feeling is that a direct reaction occurs within thirty seconds and may or may not be biphasic, that if no color develops for five minutes the reaction is indirect, while a delayed direct reaction occurs between these limits of time

The diazo color has been variously described Most authors, including van den Bergh, have termed it red (rot), but many terms, such as red-violet red orange, red-blue etc., have been used to describe the color produced Peters and Van Slyke gave the color in prompt direct reactions as blue, and went to the trouble to italicize the word, yet in another place in the text they described the color as bluish-violet, and the color in the indirect reaction as red It is true that the  $P_H$  affects the color, but in fresh serum giving a direct reaction with the original Ehrlich reagent, the color is distinctly red If Thannhauser and Andersen's formula for the Ehrlich reagent is used, the color tends to be more violet or blue in direct reactions, but red in all other types

The mathematics of the test has proved to be a stumblingblock At the outset, van den Bergh and Snapper used a standard made as follows 5 mg of pure bilirubin was dissolved in 100 cc of chloroform, and 1 cc of this solution was evaporated to dryness, this, in turn, was redissolved in 10 cc of alkaline alcohol to which 2.5 cc of Ehrlich's reagent was added Curiously enough they considered this a 1:200,000 solution instead of a 1:250,000 solution, as it actually was, and this error was carried down through all the literature until it was pointed out by Hunter in 1930, and even since then practically all authors have continued the error Thus, it appears that all recorded determinations on this basis alone are 20 per cent too high, because all artificial standards, with the exception of those made by White, who profited by Hunter's discussion, have been made by comparison with this original formula

The effect of the precipitate on the ultimate color, and hence on the final result, has been a matter of considerable controversy In the original technique the volume of the precipitate was deducted from the total volume of the supernatant liquid and was therefore taken into the calculation Evidently, however, van den Bergh and Snapper forgot to take into consideration the dilution effected by the addition of 0.25 cc of Ehrlich's reagent, which in effect diluted the final solution 20 per cent In 1918, van den Bergh corrected this error, but stated that the precipitate was not sufficiently great to

be considered and thus his dilution was considered to be 1.5. It has been shown by subsequent authors that when alcohol only is used as a precipitating agent, the factor of dilution is approximately 1.4 although this varies with each individual test. Thannhauser and Andersen considered their dilution as being 1.6, and failed to take into consideration the rather large bottom layer which apparently contains little if any color. Subsequent authors, such as Hall, have not agreed with this position, and have found that the dilution is actually only about 1.3 by this technique. The most careful students have advised measuring the volume of the supernatant liquid and using this as the factor of dilution. Of authors of recent textbooks, this plan has been adopted by Hawk and Bergem in the latest edition of their work and by Trumper and Cantarow, but the volume of the uncolored bottom layer has been ignored by Peters and van Slyke and by Todd and Sanford, while a compromise of considering the dilution 1.4 is accepted by Harrison, Osgood and Haskins, Kolmer and Boerner, Gladwohl, and others.

Although it is of the utmost importance to know by how much the serum is diluted in order to make the correct calculation, it is also important to know whether some of the bilirubin or some of the color is carried down in the precipitate. Concerning these two points there still remains obscurity but on the basis of information available it would seem that the best calculation would be based on using the volume of the supernatant liquid as the factor of dilution, especially if Thannhauser and Andersen's method is followed. Many authors ascribe procedures to Thannhauser and Andersen which they did not use, thus, it is not uncommon to find authors recommending use of the weaker Ehrlich's reagent, changing both the amounts and the order in which reagents are added, and ignoring the fact that Thannhauser and Andersen used the ammonium sulphate only in cases of obstructive jaundice, with high content of bilirubin.

The error in the original standard, the usual errors in calculating the results, and the natural evaporation of the alcoholic solution have all tended to make too high values for serum bilirubin. With these errors varying as they do, there is no wonder that little agreement exists as to the normal range of concentration of serum bilirubin in man. The values as given range almost from infinitesimal amounts to the higher figure of 2 mg. per cent, as given by Greene and his coworkers. Obviously, for a correct understanding of such values, the exact technique and calculation must be clearly noted.

As important as the quantitative determination of bilirubin would appear, the type of reaction seems to be more important. It is quite clear that while van den Bergh's original statement that a direct reaction occurs in the presence of obstructive jaundice and an indirect reaction occurs with the serum of normal persons and with that of patients with hemolytic icterus is true in general, it is equally true that this relationship does not always hold. Mann and Magath have clearly demonstrated the fact that bilirubin is formed outside of the liver, and this bilirubin gives an indirect reaction. There is considerable evidence to support the view that with the serum of man a direct reaction is not obtained unless the bilirubin has passed through the polygonal

cells of the liver, and in spite of the fact that the reactions are different in these types of bilirubin, Magath and Sheard, and others, have shown that the bilirubin itself is the same substance, or at least gives the same spectrophotometric curve and the same general reactions. Just how the bilirubin that gives the indirect reaction is bound up is not known, but it might be suggested as a logical view, that the bilirubin which reacts indirectly, is associated in some way with serum colloid, that it is colloidal in nature, that it is changed to a crystalloid form as it passes through the polygonal cells of the liver, and that this is the factor which converts the bilirubin into a state which yields a direct reaction.

In the light of this explanation and other known facts, the type of reaction becomes markedly significant and indicates that when a direct reaction is present there is injury to the polygonal cells in the liver. Contrary to the usual conception, one often sees direct reactions when the quantity of bilirubin in the blood is small, for the hepatic cells may have been definitely injured either by mechanical or physiologic obstruction. The emphasis in the literature, in the past, has been made on the type of jaundice present as evidenced by the type of reaction. In the light of present knowledge, this appears unfortunate, for the qualitative test is far more useful as an indication of hepatic injury than it is as an indication of the type of jaundice, many direct reactions being noted when there is no mechanical obstruction. A reason for missing these direct reactions when the total quantity of bilirubin is small is found in the usual method of performing the test, and may be avoided by making a ring test of the reaction on very fresh serum.

The quantitative determination of bilirubin has its greatest value in following the course of patients before and after operation or some other form of treatment, and a falling value for bilirubin often will indicate a good prognosis or a suitable time for surgical intervention before any other test or observation is definite enough for such a decision. On the other hand, a rising value for bilirubin should be considered with apprehension, and following an operation on the biliary tract indicates a grave prognosis.

It can be seen that there is still ample opportunity for scholarly investigation of serum bilirubin, permanent and more correct standards need to be provided, the effect of precipitating reagents needs to be studied, and the optimal conditions for development of color before and after addition of alcohol need to be more carefully examined. In spite of all these deficiencies, the test, if even reasonably well carried out, will yield extremely valuable information and must still be considered the most useful single laboratory test for hepatic function.

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—T B M

## CHECKING THROUGH

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WHY," said I to the detail man, "should I use your stuff when I can write just as good a prescription for nonproprietary items?"

"It's a matter of checking through," said he. "The care expended by a manufacturer in control of quality and uniformity means something to you and to your patients." Perhaps I did not look impressed, perhaps he was tired and the day hot—anyhow he added vehemently, "Go see for yourself, if you don't believe me."

That's how it started. I mentioned it to a certain medical editor. "Go," he told me, "to Bridgeport. There is a laboratory there that is outstanding and unusual. Don't ask for an appointment, just drop in and talk to them." So one bright morning I was greeted by Dr. E. C. Fanto, Chief of the McKesson and Robbins Research Laboratories. Dr. W. J. Horn, Mr. J. M. Leask and Dr. N. Michailovsky, his associates and their staff of six assistants.

"Checking through," smiled Dr. Fanto, "of course we check through, but the work starts much earlier than that. As a matter of fact in this laboratory we have done work during the past few years that is of purely scientific or protective interest and from which we do not expect a return. Part of this has dealt with the possible effect of impurities in certain drugs on general well-being, and on the formation of tumors. Part of it has dealt with the action of drugs on tumors. Part of it has been toward the development of new and improved processes and methods. All of these things have cost probably over \$100,000, which are a contribution to the scientific side of our work, a protection for the physician who prescribes and the patient who uses our products."

The laboratories occupy the entire fourth floor of the McKesson and Robbins plant in Bridgeport. Completed in 1930, these laboratories are supposed to represent the last word in equipment and in housing conditions for the animals used in the various controls and tests.

An unusual feature is the miniature manufacturing plant in which small test quantities of various medicaments may be made up under the same conditions that exist in actual commercial production. These are naturally quite often at variance with the conditions encountered in strictly laboratory work. Thus when a product is put into commercial production at the Bridgeport plant there are no uncertainties as to procedure or final perfection. Every step has been thoroughly worked out and tested in the miniature plant.

"No wonder you can do good work," I told Dr. Fanto, "with all this to work with."

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\*Publisher's Note. This article is being presented to give the readers of this journal an idea of the scientific work being done in the laboratories of modern pharmaceutical manufacturing firms and an appreciation of the scientific spirit pervading such institutions for the protection of physicians and their patients.

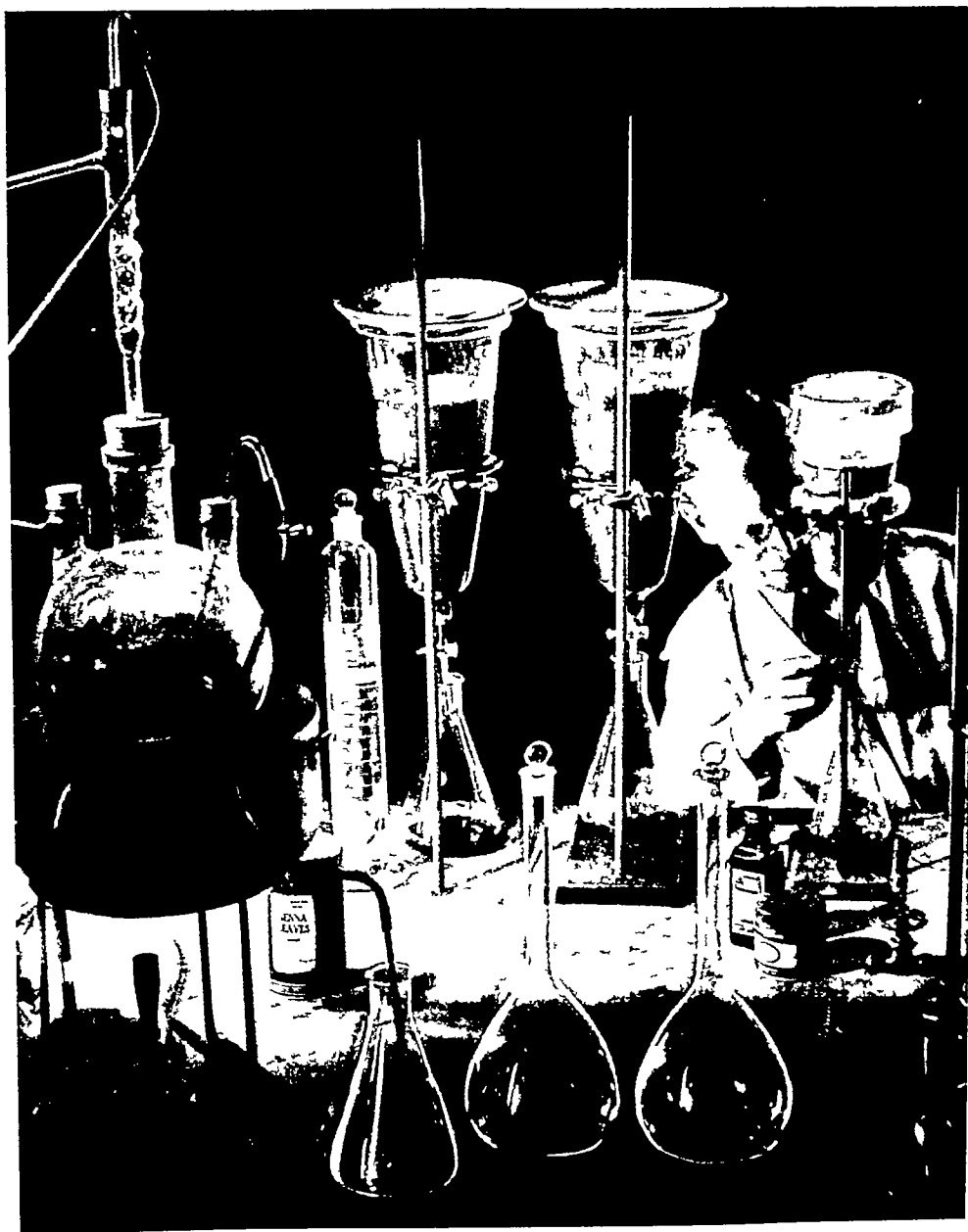


Fig 1—Method used for extracting the active principle from various drugs. The process is known as percolation which is the extraction of soluble matter from its admixture of insoluble material by a suitable solvent. The solvent travels through the mass to be extracted removing the partially saturated solution from one surface while fresh solvent is admitted at the opposite surface.

Fluid extracts and concentrated extracts are prepared from the dilute percolate by evaporation in a still under reduced pressure to the desired volume.

This photograph shows the chemist watching and regulating the flow from the outlet of the percolator. This solution will then be transferred to the vacuum outfit shown on the left so that 1 cc of the finished extract will represent 1 gram by weight of the active principles of the drug.



"Well," he replied, "it is a help, but even before we had this plant our work was creative too"

I learned then that zinc stearate is one of the many products originated by McKesson and Robbins in their old plant and introduced by them to the medical and pharmaceutical professions. Another was the introduction of Albolene following the classical work of Sir Ambrose Lane on intestinal lubrication. This product is the finest obtainable mineral oil, put through the most exacting tests for viscosity and purity. These tests may be considered "checking through," but as I was told, the laboratory does not by any means stop there. Following the publication of certain experiments conducted at Manchester University in England indicating a belief that impure mineral



Fig 2—This picture demonstrates how Medicinal White Mineral Oil (Albolene) is investigated in the McKesson and Robbins Laboratories to conform to rigid specifications of this oil for internal use. It is standardized in regard to its lubricating properties expressed as the viscosity by measuring the relative degree of fluidity at 100° F or the flowing time in records of an empty graduated flask 60 cc content at a certain temperature to a certain mark. The behavior of this oil when exposed to cold winter temperature cooled down to 20° C is examined. Note the freezing mixture to the right. The purity of the grade of refining of this oil is controlled by certain sensitive chemical reactions to be absolutely sure that the oil represents the highest degree of refining and not to contain any oxidizable carbonizable substances or sulphur compounds.

oils possess carcinogenic properties, most careful checks were made on thousands of mice in the Bridgeport laboratories. In this work both external applications and certain forms of internal administration were employed. While the results have not been published it seems to have been established that the purest grade of mineral oil as typified by Albolene is harmless.

For years the laboratories of McKesson and Robbins have devoted themselves to development of new antiseptics, especially phenols with aliphatic radicals with a higher number of carbon atoms than previously, compounds of unusually high germicidal power and relatively low toxicity. At the an-

nual meeting of the American Pharmaceutical Association in 1929 there already was read a paper by L Himebaugh and E C Fanto, on this subject. A series of comparative experiments comparing germicidal action with toxicity were presented. This study had been carried out on the living tissue of heart muscles of eight day old embryo chicks. They were able to point out at that time that one of the germicides in question, to which the laboratory

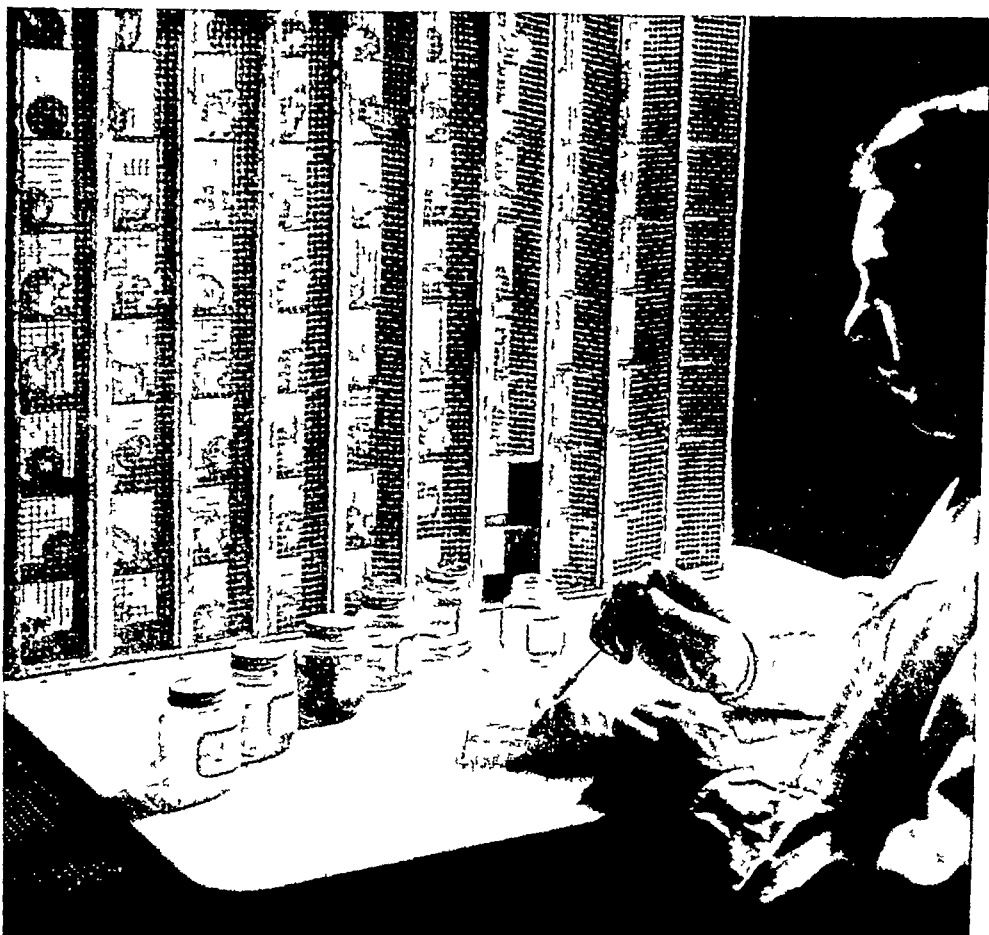


Fig 3—Investigation of certain properties of the chemicals used for preparation of compounds for external application is of great importance. Especially in view of the fact that some of the substances when not sufficiently purified or improperly treated may exert irritative or carcinogenic qualities in prolonged use. The picture shows skin painting by certain substances intended for external use and investigated for their effects through such application. These tests are often continued from 6 to 12 months before conclusions are reached.

number 801 was given, shows a tissue toxicity only 25 per cent of that of phenol. Taking into consideration the fact that this compound has a phenol coefficient of 533 against *Staphylococcus Aureus*, the toxicity to living tissue for a solution of equal germicidal power would be 0.04 per cent of that of phenol. Later tests on other animals have confirmed the relative nontoxicity of those compounds in the strengths of solutions as recommended for application.

We reached the vitamin assay laboratories under the supervision of Dr W J Horn Here lies the control for the McKesson and Robbins Cod Liver Oil and Cod Liver Oil Concentrate Here, too, the check-up starts at the very source To avoid variations, almost unavoidable in purchasing oils of different grades and origins on the open market, McKesson and Robbins acquired control of the largest Cod Liver Oil plant in the world, situated in Beigen, Norway This plant now refines nearly one quarter of the cod liver oil produced in Norway which leads the world in this commodity

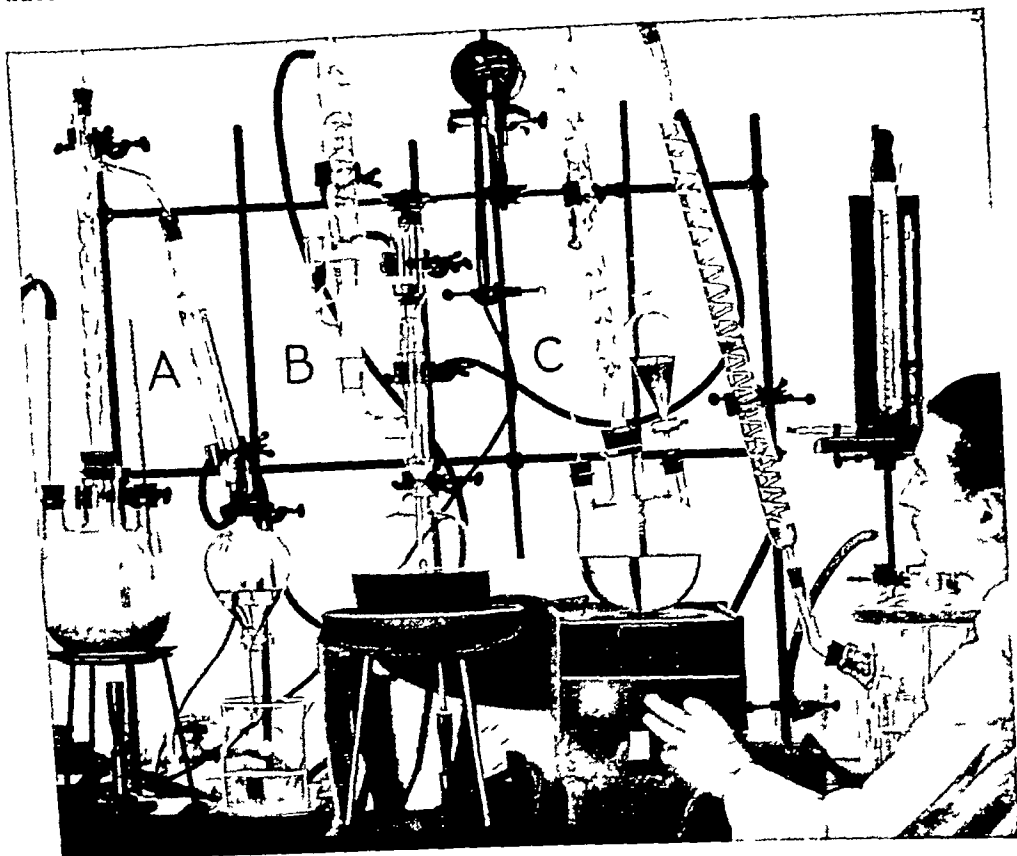


FIG. 4—The original laboratory set-up for the Organic Synthesis of certain Phenolic Germicides originated in McKesson and Robbins Research Laboratories  
Extensive research work is being done along the lines of producing powerful and very efficient antiseptics and germicides The still A at the left represents the initial step a destructive distillation the product of which is then condensed in B with other chemical compounds and the crude condensation product is finally purified by fractional distillation in C under high vacuum

The oil is produced under the most modern and scientific methods The first step is to remove the livers, wash them and place them in clean covered double walled vessels through which steam is passed, all within a few hours of the time the fish is caught This rapidity of handling eliminates unnecessary exposure to air which has the tendency to destroy Vitamin A Next the oil is filtered into galvanized cooling tanks where it clears for 12 to 14 hours Many refineries cease their work at this stage and ship the oil Not so with McKesson's Oil The crude oil is then pumped to refineries operated under a

vacuum system where the remaining blubber particles are removed and the product made nonfreezing by chilling it to  $8^{\circ}\text{C}$ . From the freezing tank the oil passes through a number of filter presses in a refrigerated room, where through an entirely new vacuum process the oil is clarified and all of the Vitamin A content retained. It is then shipped in tanks from which all air has been removed and replaced by nitrogen to prevent oxidation of vitamins in transit.



Fig. 5—The technician in the bacteriological laboratory is about to start the Food and Drug Administration phenol coefficient test on a concentrated germicide of the coal tar type (Lax-Ege). 0.5 c.c. of typhoid culture is added to one of a series of dilutions of this to determine which dilution kills the organism in ten minutes and not in five. This is found by making five- ten- and fifteen-minute transfers from the dilutions after inoculation into subculture tubes of broth and incubating these for regrowth of the organisms. The result gives a coefficient of 3.8 against *Eberthella typhi* and 1.6 against *Staphylococcus Aureus*. This particular test is an example of one of the many kinds executed in the laboratory where bacteriological research work is being carried on continuously in an attempt to perfect even more efficient germicides than are now in common use.

Before shipment each lot is biologically tested under the supervision of Professor Poulson. When the oil is received by McKesson's it is again assayed biologically for Vitamins A and D according to the methods proposed by the vitamin committee of the A. D. M. A. McKesson's medicinal cod liver oil will average 800 units and never less than 500 units of Vitamin A and 150

units, never less than 100 units Vitamin D per gram of oil when tested biologically by the above method

"Concentrates?" I asked "Liquid and tablets," smiled Dr. Horn. The liquid concentrate is made under the Marcus patent which ensures almost complete retention of the original vitamin content. It is intended primarily for infants and children where minims may be substituted for teaspoonfuls of whole cod liver oil. The tablets also prepared by exclusive methods, followed as a logical development and are standardized at a very high Vitamin A



Fig 6—This photograph illustrates one of the stages in the biological assay of Vitamin D, the examination of the tibia of a rachitic rat after treatment with cod liver oil. Rigid control and accurate data are kept on each assay so as to be sure that each package going to the consumer contains the required potency as specified on the label. The A D M A methods of assay are used.

unitage (1000 units per tablet). In addition each tablet contains a definite proportion of di-calcium phosphate.

"What is your most recent development?" was my next question.

"Well," I was told, "that is hard to say. Here as with other large pharmaceutical houses, we are constantly endeavoring to develop new ideas, products as well as technique."

I gathered for instance that at the present moment some quite extensive clinical investigation is being carried on to determine the merits of a colloidal

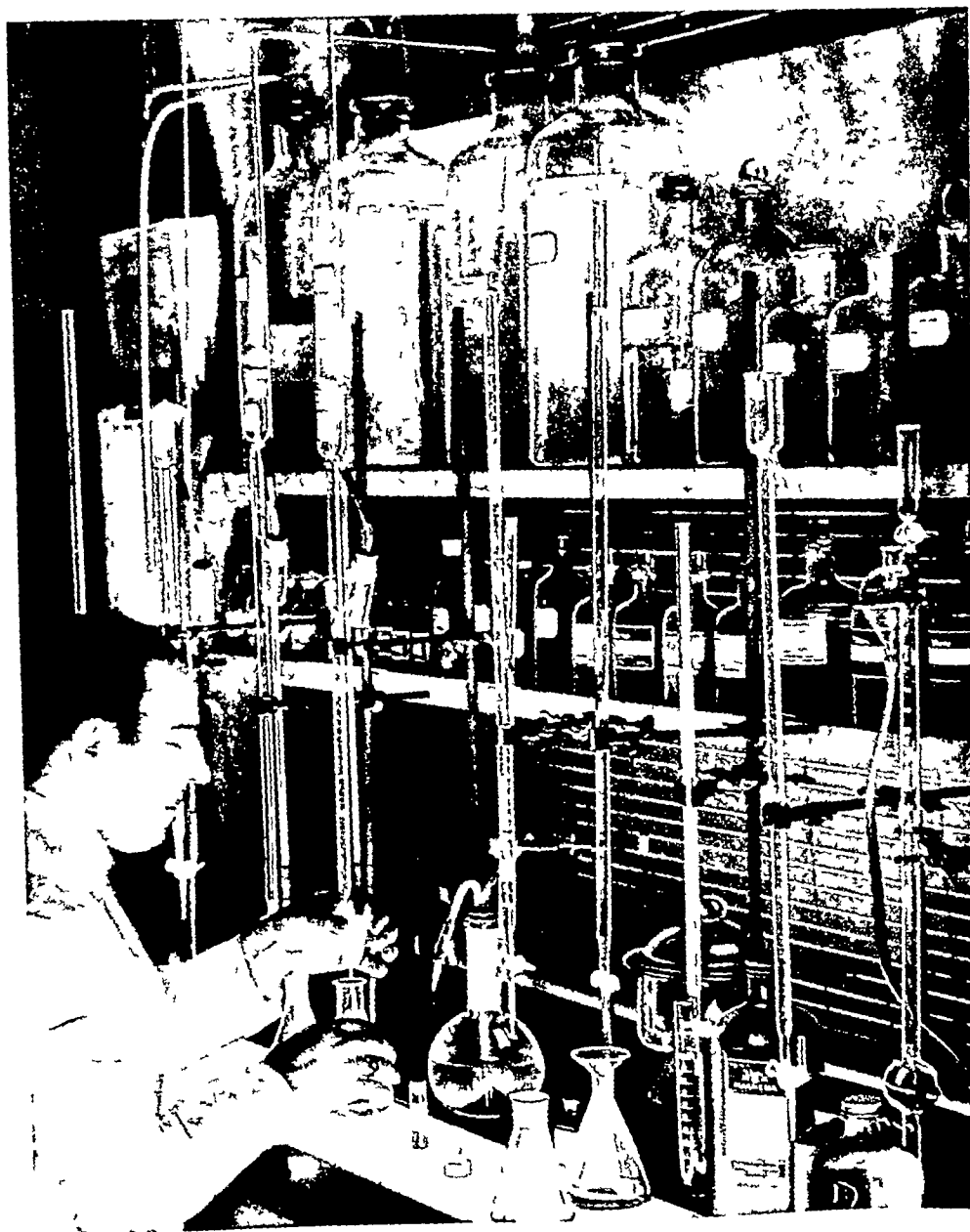


Fig 7—Volumetric Analysis. McKesson and Robbins pharmaceutical preparations are assayed according to the pharmaceutical standard methods. The picture records an up to date titration table on which by volumetric analysis strength and purity are determined using Standard Volumetric Solutions and specific color indicators.

In actual operation the solution of a proper reagent whose exact strength at the time of the determination must be known is carefully added from a burette until the completed color change indicates the reaction. The chemist in this photograph is measuring a certain amount of liquid into an Erlenmeyer flask. From the volume of the solution of the reagent consumed to produce this reaction the quantity of the substance sought is calculated.

copper morthuate in certain tuberculosis cases. This product is given by injection intravenously or directly into the cavity. This work has been going on for over a year, but as yet the product has not been offered to the profession generally.

Then there are the experimental studies, laboratory and clinical, on the effects of certain chemical compounds on tumors. These have already lasted four years, and soon, I heard, a preliminary report will be made.

Even the so called routine work of control and testing may lead to im-



Fig 8—This picture represents a laboratory replica of ointment mass as used in a bigger scale in factory production of McKesson and Robbins. To prevent any gritty particles from being left in ointments, dentifrices and salves these products are run through a system of rollers. The distance between the rollers is microscopically close so that the stream of ointment mass is compelled to pass through the rollers in a very fine layer.

proved products, new "developments" if you wish. In any event every batch of even the most "ordinary" type of pharmaceutical product goes through most careful testing processes, and, of course, each batch is numbered, listed and control samples kept.

Probably the last product to be completely developed in these laboratories is the McKesson and Robbins Copper Iron Compound. When interest in this type of medication developed, work was undertaken to find a preparation which would have a marked effect upon hemoglobin regeneration and which would be well tolerated and effective when administered orally. Realizing that



Fig 9—Developing a compound for any purpose requires a thorough study from different angles. It goes a long way before the final formula is reached. This photograph shows the process of filling the ampules prior to their sterilization with a compound for the laboratory investigation and experimentation on animals.



Fig 10—The importance of a thorough investigation of the effects produced by various chemical substances which prior to human application are studied on animals leads us to a necessity of a very accurate analysis of the effects produced. Pathological examination of changes in animal tissues due to the external or internal application of certain substances represents one of the methods by which the effects are studied. The picture shows preparation of the tissue sections for the microscopical examination in connection with the experimental work shown in Fig 3.



inorganic salts of both copper and iron are astringent and not well tolerated by the digestive processes investigations were made using combinations of these two metals in the form of organic proteid compounds in which the copper and iron were in the "masked form" Many experiments conducted upon animals showed these combinations to be nonastringent or toxic and to cause no dis-

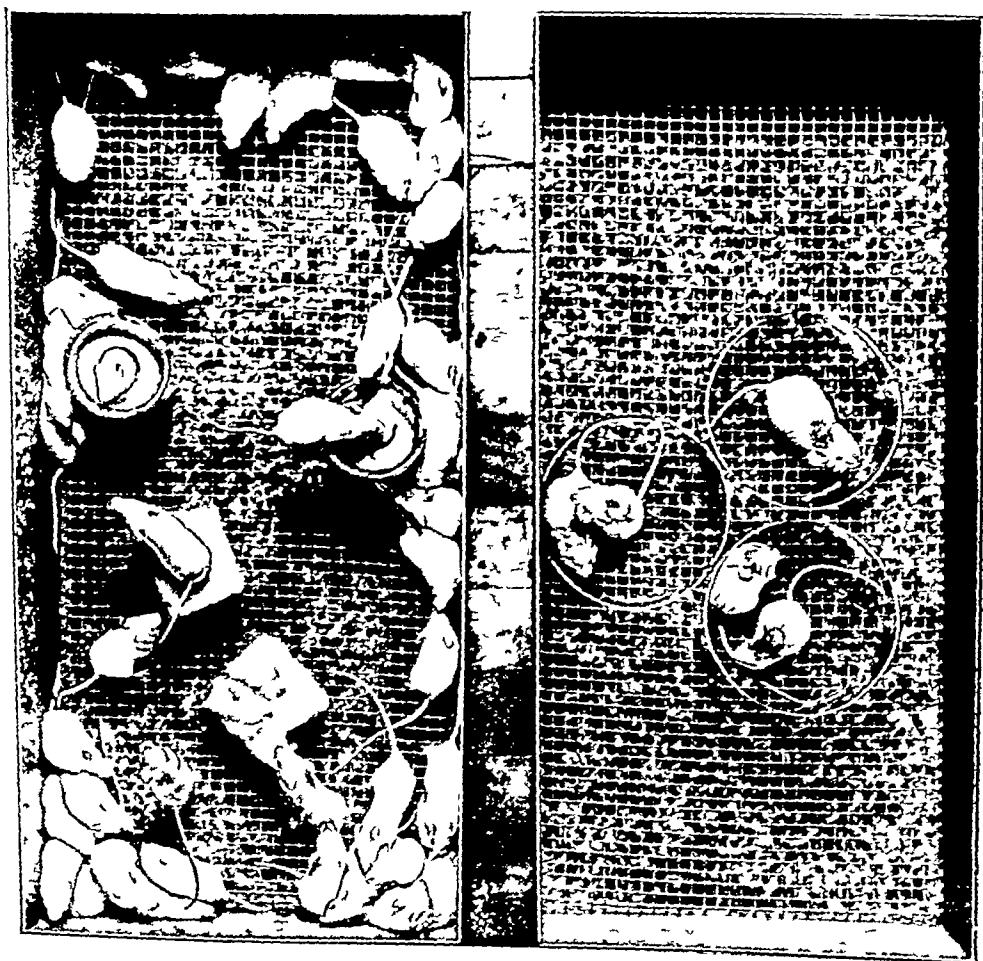


Fig. 11—Animal experimentation plays a very important part in the study and development of certain preparations This photograph offers an example of comparative toxic action produced by a highly refined product and the same product in an inferior state of purification In the right cage it will be seen that only five mice are living after a three-month test period in which the inferior product was given internally The left cage shows the large number of mice that were given the superior product, still living Both cages contained the same number of animals at the beginning of the experiment

turbances in the digestive processes The effect upon hemoglobin regeneration in animals produced by the above combinations of this sort were found to be startling In every series of experiments similar results were obtained This work was then followed by clinical tests upon humans with equally gratifying results

Each lot of *Copper-Iron Compound* is carefully standardized as to copper and iron content, the ingredients used in the manufacturing of the preparation being of the highest quality and purity obtainable

And therewith closed my visit

To me it was full of human interest and a revelation of the extent to which the important pharmaceutical manufacturers work for and with the



Fig. 12.—The chemical and physical constants of McKesson and Robbins Essential Oils, Balsams, Oleo-Resins and Vegetable Oils are thoroughly investigated. All samples are carefully examined for the presence of adulterants and other objectionable ingredients. All U. S. P. and N. F. Oils must meet the specifications given in these official publications. Determination of the optical rotation of a sample of essential oil with the polariscope is standard practice in these laboratories.

medical profession, of the amount of nonremunerative research work they do and of the scientific accuracy of their methods. It is for these reasons that I have offered it for publication supported by the excellent descriptive illustrations for which I am indebted to McKesson and Robbins.

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## CLINICAL AND EXPERIMENTAL

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### STUDIES ON THE PHYSIOLOGIC EFFECTS OF FEVER TEMPERATURES\*

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THE THERMAL DEATH TIME OF *NEISSERIA GONORRHOEAE* IN VITRO  
WITH SPECIAL REFERENCE TO FEVER TEMPERATURES

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THE ever changing therapy for disease caused by *Neisseria gonorrhoeae* is evidence of the unsatisfactory results obtained in general by clinicians. Chemotherapy and the use of specific products of the gonococcus are employed most frequently, although the use of local or general applications of heat by means of hot water or by diathermy is recommended by many physicians. There are those who induce fever in such patients by injecting foreign proteins especially sterile milk and typhoid vaccines. The literature contains many favorable and unfavorable reports on such procedures. Much progress has been made recently in the application of diathermic and radiothermic fever therapy, and therefore this method is becoming more extensively used. The lack of uniformity of results from the use of these artificially induced fevers when the thermolability of the gonococcus is considered presents an important problem for further analysis. We believe that the determination of the thermal death time of infectious agents at fever temperatures is fundamental in establishing a basis for such therapy.

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## HISTORY

Some interesting observations on the effect of fever temperatures on *N gonorrhoeae* have been reported in the literature, although the investigators were not primarily concerned with the thermal death time of the organism. Finger, Ghon, and Schlagenhauser<sup>1</sup> stated that they failed to produce an urethritis in man by injecting the gonococcus into the urethra of a patient with a fever of 39° or 40° C. However, they were always successful if the host was afebrile. Guaid<sup>2</sup> observed a patient with gonorrheal urethritis who was cured after an attack of scarlet fever. Neisser and Scholtz<sup>3</sup> stated that it was always more difficult to obtain a culture of the gonococcus from patients when they had a fever than when they were afebrile. Bogdan<sup>4</sup> described a case of gonorrheal urethritis in which the purulent discharge disappeared during an attack of pneumonia but returned after the fever had receded. Luys<sup>5</sup> noted a similar case, in which the urethral discharge subsided during an attack of mumps with a fever of 40° C but returned after the patient was afebrile. Culver<sup>6</sup> described a case of gonorrheal urethritis that recovered after having malaria four days, in which there was a maximum temperature of 40.5° C. He further states that if the body temperature is suddenly elevated from normal to 39° C, the gonococcus is destroyed. Others have observed a similar disappearance of the purulent urethral discharge during a febrile reaction. We have also observed the absence of the purulent exudate in our male patients, and likewise in those women with a profuse vaginal discharge, after treatment with a fever of 41.5° C for five hours, produced by the passage of high frequency currents through the trunk of the patient. However, during the fastigium of such fevers, there occurred a marked increase in the mucopurulent discharge from the lower birth canal, which subsided after the treatment. Boerner and Santos<sup>7</sup> heated the male urethra with "diathermy" currents, and were unable to isolate the gonococcus after ten hours at 39°, three hours at 41°, and fifty-seven minutes at 41.7° C. Ylppo<sup>8</sup> treated with hot-baths a five-year-old girl with vulvovaginitis. The bath at the beginning of the treatment was 39° C and was gradually raised to 41.5° C. After a series of eight daily treatments for one hour he was unable to isolate the gonococcus.

In contrast to these observations, Nobl<sup>9</sup> reported a series of five cases that had, superimposed on a gonococcus infection, either pneumonia or pulmonary tuberculosis with a fever of 40° C. He states that the venereal disease was not influenced. Nicoll<sup>10</sup> likewise observed three cases of gonorrheal septicemia and arthritis immediately following an attack of scarlet fever with a marked febrile reaction.

Practically all bacteriologists consider that 37° C is the most suitable temperature for the isolation and cultivation of *N gonorrhoeae*, and that temperatures above 38° C definitely injure the organism. Kiefer,<sup>11</sup> and Steinschneider and Schaffer<sup>12</sup> report that the gonococcus is killed in a few hours at a temperature of from 40° to 41° C. Wertheim<sup>13</sup> states that this organism grows well at a temperature of 40° C, and resists a temperature of 42° C.

However, this has not been confirmed by the observations of other investigators. Ungeimann<sup>14</sup> observed several strains that resisted 41° C for ten hours, while three or four strains survived 52° C for seven hours. Santos<sup>15</sup> reported that he was able to isolate the gonococcus from pus if it was not kept longer than forty-five minutes at 45° C or five minutes at 50° C. Koch and Cohn<sup>1</sup> believe that this microorganism is more resistant to fever temperatures *in vivo* than *in vitro*. They base their opinion upon the fact that the infection resists a temperature of 40° C for several days in man, during the course of an acute infectious disease.

Many other observations have been made on the thermal death time of the gonococcus, but these are concerned principally with the length of time required to kill the organism when subjected to from 45° to 60° C, which are temperatures that cannot be tolerated by man.

#### METHOD

The following experiment was designed to establish a basis for the fever treatment of patients infected with *N. gonorrhoeae*. We desired to determine the length of time required by such temperatures as 39°, 40°, 41°, 41.5°, and 42° C to destroy pure cultures of the gonococcus *in vitro*.

Fifteen strains were obtained for this study, eight of which (Nos. 1 to 8 inclusive) were sent to us through the courtesy of Dr. John Torrey of the Cornell Medical School, New York City. All were isolated in 1920 with the exception of No. 8, recovered in 1922, from cases of chronic urethritis in the male. These strains have been under artificial cultivation from ten to twelve years. During this period they have been kept on Torrey's semisolid, vitamin medium that he described in 1922.<sup>17</sup> We shall designate these strains as "old." Seven cultures (Nos. 9 to 15 inclusive) were isolated from one to four months previous to these studies by Mrs. Alice Leahy, of the Bacteriological Laboratories of Strong Memorial and Rochester Municipal Hospitals. The source of these cultures was variable—joint fluid, male urethral discharge, or cervical and vaginal discharges (see Table I). The recently isolated strains of gonococci are designated as "new." These cultures were isolated and kept on a glucose, ascitic blood agar, prepared as follows: To 100 c.c. of Douglas' agar<sup>18</sup> with a  $P_H$  of 7.5 to 7.6 was added 25 c.c. of ascitic fluid, 5 to 8 c.c. of defibrinated rabbits' blood, and 1 per cent glucose. Ten days before immersing the cultures in the water-baths to determine their thermal death time, all were transferred to a glucose, ascitic, fluid broth, prepared by adding to 100 c.c. of a meat extract broth 25 c.c. ascitic fluid and 1 per cent glucose. After two or three generations in this broth, a 100 c.c. flask of the same medium was inoculated with the various cultures and allowed to incubate at 37° C for forty-eight hours. The cultures were thoroughly shaken to break up the granular colonies and to insure a uniform suspension.

With a 20 c.c. Luer syringe, amounts of 1½ c.c. of the culture were then transferred to small glass vials, sealed, labeled, tied to dental film holders, and immersed in a series of water-baths. The baths were set at temperatures of 37°, 39°, 40°, 41°, 41.5°, and 42° C. The variation of the temperature of

the baths is no greater than 0.002° C. A complete description of the baths used for this work is published elsewhere.<sup>19</sup> At hourly intervals for thirty hours, one vial was removed from each bath and unviability of the organism was tested in the following manner. After breaking the stem of the vial with a file the contents of the vial were removed with a sterile Wright pipette and placed in a sterile tube. One-half cubic centimeter was pipetted onto the slant of a tube of glucose, ascitic blood agar, and the stoppers sealed in with paraffin. One-tenth cubic centimeter was then added to 100 c.c. of a sterile, physiologic salt solution for plating. The medium used for plating was a

TABLE I  
SOURCE AND DATE OF ISOLATION OF *GONOCOCCUS* CULTURES USED IN EXPERIMENT

NUMBER	SOURCE	SEX	DATE OF ISOLATION
1	Urethra	Male	1920
2	Urethra	Male	1920
3	Urethra	Male	1920
4	Urethra	Male	1920
5	Urethra	Male	1920
6	Urethra	Male	1920
7	Urethra	Male	1920
8	Urethra	Male	1922
9	Cervix	Female	1931
10	Joint	Male	1931
11	Joint	Male	1931
12	Urethra	Male	1931
13	Cervix	Female	1931
14	Joint	Female	1931
15	Cervix	Female	1931

glucose, ascitic fluid agar, prepared by adding 1 per cent glucose 25 c.c. of ascitic fluid and 5 c.c. of defibrinated rabbit's blood serum containing a few cells, to 100 c.c. of Douglas' agar with a  $P_H$  of 7.6. Dilutions of 1-1,000 and 1-10,000 were then made in the usual manner. As the thermal death time was approached in subsequent experiments lower dilutions were used for plating and finally 0.10 c.c. of the culture to be examined for viability was plated directly. A slightly different medium was used for plating some of the cultures. This medium, which is a hemoglobin serum agar and is extensively employed in England for the cultivation of *N. gonorrhoeae* was prepared as follows. To 100 c.c. of liquefied 2 per cent digest agar was added 15 c.c. of sterile horse serum and then 1 c.c. of laked horse blood cells. The serum and hemolyzed cells were added after the agar had cooled to a temperature of 50° C. or below. The red blood cells were laked with chloroform.

The cultures were incubated at 37° C. The plates were counted forty-eight hours after they were made, while the tubes were examined for growth in seventy-two hours. The tubes and plates served as an excellent check on each other. The tubes gave us a more favorable medium and better atmospheric conditions for growing the gonococcus while making plate counts gave us an opportunity to study the rate the gonococci were destroyed.

The number of gonococci per cubic centimeter in the broth cultures at the time the experiment began varied from 1,200,000 to 25,000,000. We ob-

served that this variation in the number of organisms in the suspensions to be heated in the water-baths was not as important a factor as the age of the suspension. A forty eight hour growth was found to be the most resistant under the conditions with which we worked.

The thermal death time of the cultures was checked in two ways. First, the same strain was used in all the baths, hourly examinations being made. With this method it was difficult to work with more than two or three different strains at one time. Later several strains were exposed to the temperature of only one bath and examined hourly. Determinations of the number of hours required to destroy the gonococci at the various temperatures were made of each strain at least three times.

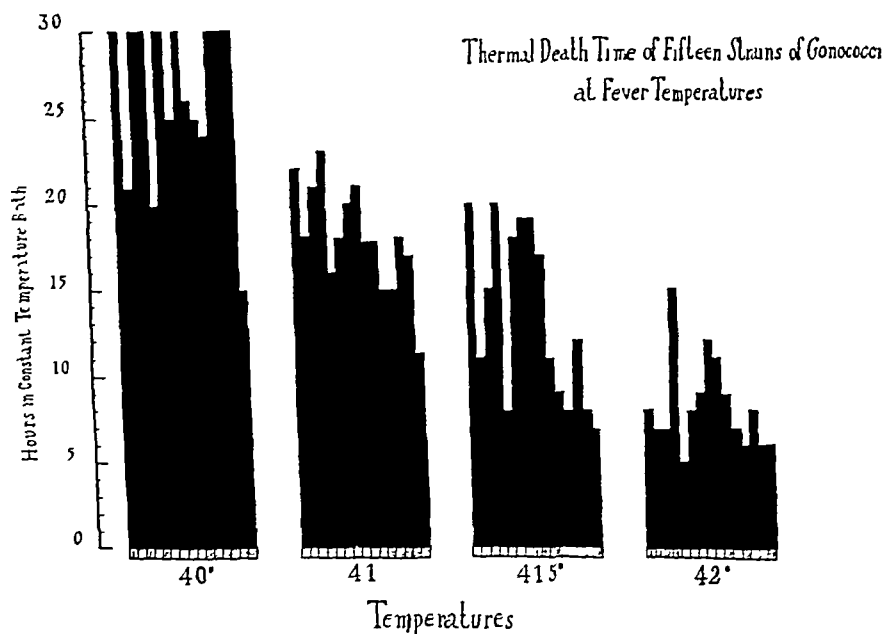


Fig. 1—Showing the thermal death time of the fifteen strains of gonococci. Black lines represent death of all organisms.

# RESULTS

The bath set at 37° C. was used for the control temperature. The vials containing these cultures were examined at hourly intervals for the length of time of the experiment, and, in general, the number of gonococci continued to increase throughout the thirty hour period. This indicated that there was sufficient food available for the organisms to grow and that atmospheric conditions and  $P_H$  values were satisfactory during the experimental period.

It was observed that 39° C. apparently had some injurious effect on the growth of the organisms because the counts showed practically no change in the original number of organisms in the suspension before exposure.

The results of the lethal effects of the fever temperatures 40°, 41°, 41.5°, and 42° C. are shown in Fig. 1. Inspection of these data gives three general

impressions. The first is that the thermal death time of the gonococcus at these fever temperatures is very variable. Second, the thermal death time of some of the strains is within the limits of fever temperatures that can be produced safely in man. Third, the cultures of gonococci that have been under cultivation for several years are, on the average, more resistant to fever temperatures than those recently isolated.

A temperature of  $40^{\circ}\text{C}$  for thirty hours failed to destroy five (1, 3, 4, 6, and 8) of the eight "old" strains, while only three (12, 13, and 14) of the recently isolated strains of gonococci were still viable at the end of this period. The other three strains (2, 5, and 7) under cultivation for approximately twelve years were not viable after twenty-one, twenty, and twenty-five hours respectively. The other four recently isolated strains (9, 10, 11, and 15) required twenty-six, twenty-five, twenty-four, and sixteen hours respectively at  $40^{\circ}\text{C}$  to obtain death of all of the organisms.

At  $41^{\circ}\text{C}$ , twenty-three hours was the longest period of time required to destroy all of the gonococci (strain 4 "old"). Eighteen hours was the maximum exposure at this temperature necessary to kill all cells in any of the cultures in cultivation for only a short period of time (strains 9, 10, 13 "new"). The shortest thermal death time for the "old" strains at  $41^{\circ}\text{C}$  was sixteen hours (strain 5), while that of the "new" strains was eleven hours.

A fever of  $41.5^{\circ}\text{C}$  for five hours artificially induced by high frequency currents or by short radio waves (30 meters) is a procedure that has been used in this clinic for the treatment of subacute and chronic gonorrhea. We had previously determined that this is the maximum temperature that can be employed for this length of time safely in fever therapy. Occasionally a patient's rectal temperature has registered  $42^{\circ}\text{C}$  during the course of an artificial fever, but for only short intervals. In one or two instances the fever has been prolonged for seven hours, if the patient was in good physical condition at the end of five hours. Therefore, this is a critical temperature in these studies on the thermal death time of the gonococcus. At  $41.5^{\circ}\text{C}$  a heating for twenty hours in the water-bath was necessary to obtain death of all the organisms in two "old" strains (1 and 4). Seventeen hours' exposure was required to kill a "new" strain,<sup>9</sup> which is only one hour less than that needed at  $41^{\circ}\text{C}$ . However, the other "new" strains were much less resistant at  $41.5^{\circ}\text{C}$ , and showed a shorter thermal death time, varying from seven to twelve hours at this temperature. The shortest exposure required to kill all organisms in the "old" strains was seven hours (strain 5). However, the majority were more resistant.

Strain 4 "old" was killed in fifteen hours at  $42^{\circ}\text{C}$ , but this culture was the most resistant of any of the "old" or "new" strains. The longest exposure at this temperature necessary to cause death of all the gonococci in the "new" series was eleven hours. However, six hours destroyed all the organisms in three of the "new" strains, which was the shortest thermal death time for this group at this temperature. Strain 5 "old" was killed in five hours. It will be noted that this is the least resistant strain of all of those under cultivation for twelve years. At all the fever temperatures its thermal



death time was similar to the more recently isolated strains. During the time we have studied this strain it has never grown so luxuriantly as the other "old" strains.

In the series of "new" cultures are two strains, 11 and 12, isolated respectively from a knee joint and from the urethra of the same patient. It is interesting to note that at 40° C the urethral strain was more resistant than the joint strain, the former being still viable at thirty hours, while the latter was killed in twenty-four hours. At 41° C the thermal death time was identical, while at 41.5° and 42° C the joint strain was more resistant, requiring one hour more to obtain death of all the organisms than did the strain isolated from the urethra.

TABLE II

RESULTS OF PLATE COUNTS MADE ON GONOCOCCUS CULTURE NO 15 AT FEVER TEMPERATURES

HOURS	37° C	39° C	40° C	41° C	41.5° C	42° C
0	3,100,000	2,400,000	3,800,000	3,400,000	2,120,000	4,780,000
1				2,190,000	258,000	312,000
2	2,200,000	6,100,000	400,000	220,000	640	24,000
3				56,000	720	120
4	3,330,000	contam		2,000	580	176
5				6,000	84	9
6	1,168,000	2,090,000	360,000	1,600	14	
7				3,360	20	
8	2,650,000	3,030,000	760,000	380		
9				contam		
10	2,400,000	1,100,000	11,000	412		
11				14		
12	2,520,000	4,570,000	26,000			
13						
14	4,160,000	5,200,000	11,000			
15						
16	3,420,000	720,000	42			
17						
18	6,120,000	3,800,000				
19						
20	8,600,000	1,230,000				

Studies of the plate counts made on several of the cultures used in this experiment indicate that at temperatures of 41.5° and 42° C, 87.8 per cent and 93.4 per cent respectively, of the gonococci were killed during the first hour (see Table II). At 41° C, 35.5 per cent were destroyed during the first hour, and 93.5 per cent by the end of the second hour. Four, two, and two hours' exposure at 41°, 41.5°, and 42° C respectively, caused death of 99.9 per cent of the gonococci in the above culture (No 15), which results are representative of the death curve of the other strains examined. At 40° C, 89.4 per cent died during two hours' exposure, while 95.2 per cent were dead at the end of six hours' exposure and 99.7 per cent were killed in ten hours.

## DISCUSSION

The  $P_H$  of the broth cultures was determined when they were forty-eight hours old, at the time they were immersed in the water-baths, and after a period of thirty hours at the various temperatures. The  $P_H$  was determined by the colorimetric method, and Table III gives the results obtained on one

'new' and on one 'old' strain. It is evident from this table that the change in  $P_H$  was not great enough to be an important factor in the cause of death of the gonococci. Inasmuch as the control cultures at  $37^\circ \text{C}$  usually showed an increase in the number of microorganisms during the first fifteen or twenty hours of the experiment, evidently enough food was available in the glucose ascertic broth medium to support growth. Therefore, by eliminating a marked change in the  $P_H$  factor and by insuring a sufficient amount of food, the heat from the water-baths set at fever temperatures must have been responsible for their death.

At temperatures of  $41^\circ$ ,  $41.5^\circ$  and  $42^\circ \text{C}$  autolysis of some of the gonococci occurred from one to two hours before death of all the organisms. This was indicated by a clearing of the medium, and also by making and gram staining smears from the culture at the thermal death time. Autolysis was not observed at  $39^\circ \text{C}$ , and was only slight after about twenty hours at  $40^\circ \text{C}$ . At the higher temperatures there was not always complete autolysis.

TABLE III

RESULTS OF DETERMINATION OF  $P_H$  OF FORTY EIGHT HOUR CULTURE OF TWO STRAINS OF *N. GONORRHOEA* BEFORE AND TWENTY FOUR HOURS AFTER EXPOSURE TO THE FEVER TEMPERATURES

STRAIN	TIME	$37^\circ \text{C}$	$39^\circ \text{C}$	$40^\circ \text{C}$	$41^\circ \text{C}$	$41.5^\circ \text{C}$	$42^\circ \text{C}$
6	$P_H$ Before	7.5	7.8	7.5	7.8	7.8	7.8
	$P_H$ 30 Hours	7.5	7.7	7.7	7.7	7.5	7.8
9	$P_H$ Before	7.5	7.5	7.5	7.8	7.8	7.8
	$P_H$ 30 Hours	7.6	7.6	7.6	7.7	7.6	7.8

The lethal effect of fever temperatures on *N. gonorrhoeae* in clinical cases is a controversial matter. Some observers have reported that during naturally occurring, or induced, fevers symptoms of the disease have subsided while others state that body temperatures above normal,  $39^\circ$  to  $41^\circ \text{C}$ , have not been of therapeutic value or have caused only a temporary cessation of inflammatory discharges. The variation of the resistance of different strains to heat may be the explanation for such conflicting reports. A study of temperature gradients in the normal human body demonstrated that the temperatures of the urethral meatus and anterior urethra are from  $1^\circ$  to  $4^\circ \text{C}$  lower than the rectal temperature. We have observed that during a fever a similar gradient persists unless the temperature of the environment about the penis is maintained at or above the level of the elevated body temperature.

Although not all the organisms were killed in five hours at  $41.5^\circ \text{C}$  it is significant that 99.9 per cent of them were destroyed in the first two hours of exposure to this temperature. Since the virulence of *N. gonorrhoeae* is difficult to determine experimentally we have no method to find out if the 0.1 per cent of the organisms remaining viable were still pathogenic. It is evident that in vivo some injury other than that due to heating occurs to the gonococcus, making it more susceptible to destruction by the normal defense mechanism of the body. Clinically we have observed 'cures' re-

sulting from such a fever as  $41.5^{\circ}\text{C}$  for five hours, when we were unable to obtain an *in vitro* thermal death time for a similar exposure.

Plate cultures made from the cervix of cases of chronic gonorrhea before, during, and after artificial fever therapy demonstrate the effect of a temperature of  $41.5^{\circ}\text{C}$  for five hours on the gonococcus. Many colonies of gonococci were present in smear plate cultures immediately before the treatment, while after five hours exposure no colonies of the gonococcus could be found. This result may be due to the excessive secretion of the cervical glands during the treatment which washed the gonococci from the surface of the cervix. The cultures were made by inserting a sterile swab into the external os of the cervix and then streaking the exudate on blood glucose ascitic agar. Other types of bacteria, especially staphylococci, diphtheroids, and large, unidentified gram-positive rods, were not affected to any great degree by such fevers.

The variability of the resistance of the strains to the fever temperatures was anticipated, especially at the lower temperatures ( $40^{\circ}$  to  $41^{\circ}\text{C}$ ), when a comparatively long exposure was required. At least three determinations were made on each strain and invariably the thermal death time was identical. In some instances a variation of one hour longer or shorter thermal death time was observed for the necessary increased exposure at  $40^{\circ}$  and  $41^{\circ}\text{C}$ . The greater resistance of the "old" strains may be explained upon their better adjustment to artificial cultivation. Many of the different determinations were made on the same culture at intervals of from three to four months. Since practically no change in the thermal death time was noted in such strains during this time, it is evident that the greater resistance of the cultures to the heating is acquired slowly and after a comparatively long period on artificial media. That the gonococcus is more resistant to fever temperatures *in vivo* than *in vitro* is doubted. The abnormal environment resulting from artificial cultivation cannot be more injurious to the gonococcus than the natural defensive agents of the body, other than fever.

#### SUMMARY AND CONCLUSIONS

- 1 The thermal death time of fifteen strains of *N. gonorrhoeae* was determined *in vitro* at fever temperatures of  $30^{\circ}$ ,  $40^{\circ}$ ,  $41^{\circ}$ ,  $41.5^{\circ}$ , and  $42^{\circ}\text{C}$ . Seven strains had been under cultivation for twelve years and one strain for ten years, while the remaining seven strains were isolated from one to four months prior to beginning the experiment.

- 2 The resistance of the strains of gonococci examined to the fever temperatures was variable. The cultures that were isolated ten and twelve years ago had, on the average, a longer thermal death time than the recently isolated cultures.

- 3 At  $39^{\circ}\text{C}$  there was little, if any, effect on the growth of the organism. At  $40^{\circ}\text{C}$  about 99.7 per cent of the organisms were killed by ten hours' exposure, while death of all of the cells was not obtained at thirty hours in eight of the strains.

4 At 41° C, 99 per cent of the gonococci were destroyed in from four to five hours' exposure, while death of all the organisms required from eleven to twenty-three hours

5 At 41.5° and 42° C, 99 per cent of the gonococci were rendered non-viable in two hours. The remainder were killed at 41.5° C from seven to twenty hours, while at 42° C the thermal death time varied from five to fifteen hours. In all instances the recently isolated cultures, with the exception of one "old" strain, showed the least resistance to 41°, 41.5°, and 42° C

6 The *in vitro* thermal death time of *N. gonorrhoeae* is short enough at 41°, 41.5°, and 42° C to suggest artificially induced fever as a valuable aid in the treatment of disease due to this organism. It is doubtful whether complete sterilization by heat can be obtained always from a single artificially induced fever of five hours' duration

We wish to express our appreciation of the assistance and advice given by Dr Stanhope Bayne Jones, Professor of Bacteriology, and for services rendered by the Department of Bacteriology

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# BIOCHEMICAL STUDIES ON THE MECHANISM OF PHENYLCINCHONINIC INTOXICATION\*

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## I

### APPLICATION OF A NEW FUNCTIONAL LIVER TEST TO PHENYLCINCHONINIC INTOXICATION IN ANIMALS

IN THE course of the last few years, a number of fatal cases of phenylcinchoninic intoxication in human beings have been observed (most of them after excessive doses or after unduly prolonged administration of the drug), manifesting themselves as yellow *atrophy of the liver*. It seemed therefore interesting to establish, whether it may be possible to detect by the chemical examination of urine some signs of liver injury in animals during phenylcinchoninic intoxication.

We recently published a paper<sup>1</sup> dealing with "aromaturation," sometimes depending on severe liver injury, and we, therefore, tried to profit from this new method for the above mentioned purpose. It is generally known, that the Millon test is characteristic for phenol, as well as, for phenolic substances (such as tyrosine), and that under pathologic conditions some Millon substances occasionally appear in the urine. We tried to study the conditions which may be responsible for this occurrence. The substances, yielding the Millon test, may be separated into two groups, so far as they are either soluble or insoluble in ether. It is easy to extract with ether certain acids derived from tyrosine from the urine and to estimate them colorimetrically. But we met with considerable difficulties when we tried to make quantitative researches on the bulk of the Millon substances in the urine, which are insoluble in ether. These phenolic substances belong to a group of certain high molecular and unidentified substances in the urine, which are derived from proteins and known as oxyproteic acids. Among these oxyproteic acids there are, occasionally, appreciable quantities of phenolic substances, which in this case are responsible for the Millon test of the urine.

Several years ago, one of us,<sup>2</sup> together with his students, evolved a method for the determination of the tyrosine content of proteins based on the Millon test. The idea of this method is, that from protein hydrolysates interfering substances are removed by phosphotungstic acid, the excess of this reagent, then, by quinine, and the excess of quinine by sodium hydrate. The phenolic substances, contained in the last colorless filtrate, may then be estimated colorimetrically by way of the Millon reaction, using a 0.1 per cent. solution of tyrosine as a standard. Boiling is avoided, the color develops on standing at room temperature in the course of forty-five minutes.

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We found that the most convenient way to remove all those urinary substances interfering with the Millon test,\* is to precipitate the urine in the same manner as is usual in the determination of urea by the method of Morner-Sjoquist † The barium containing precipitate is separated and then disintegrated by 10 per cent  $H_2SO_4$ .  $BaSO_4$  is removed by filtration and the filtrate then treated like a protein hydrolysate ‡ For the final colorimetry a Bioklett colorimeter was used

We examined many urines by this method and we found that this method is a test for severe injuries of the liver (such as icterus gravis, yellow atrophy of the liver, phosphorus poisoning etc.) and for increased desintegration of body proteins (for instance in advanced pulmonary consumption)

We took advantage of this new method, revealing severe disturbances of the liver, in order to establish, *whether an increased output of phenolic substances, contained in the oxyprotein fraction, may be observed in rabbits poisoned by large doses of phenylcinchoninic acid (cinchophen) or the ester of paramethylphenylcinchoninic acid (tolysin) -*<sup>4</sup>

We performed experiments on seven rabbits (a) subacute cinchophen poisoning death after five weeks, (b) subacute tolysin poisoning, death after nineteen days, (c), (d) (f), (g) subacute tolysin poisoning, death after three or four days, (e) mild tolysin poisoning, survived

The result of these experiments was negative. Only once was a temporary occurrence of a phenolic substance in the urine ascertained. We may conclude therefore that *neither in 6 cases of acute or subacute tolysin poisoning in rabbits nor in one case of subacute cinchophen poisoning did the liver seem to have been severely injured*. Perhaps it will be possible to get positive results on animals in cases of chronic intoxication, when prolonging the experiments to a duration of several months or using a more delicate liver test, enabling us to detect initial liver changes as possible precursors of severe liver injuries and of acute yellow atrophy. Besides we saw the appearance of Millon bodies (corresponding to the quantity of 15 to 30 mg. per cent) in the urine of a hyperthyroidized rabbit in a case of subacute cinchophen poisoning, not immediately, but only a month after the last injection of cinchophen (0.4 gm. p.k.). The animal was killed soon afterward. But careful examination of the liver (Professor Carl Steinberg) did not reveal any histologic changes.

## II EXPERIMENTS CONCERNING THE ALLEGED POSSIBILITY OF CINCHOPHEN DETOXICATION BY A DIET ABUNDANT IN CARBOHYDRATES

Many years ago one of us (Fuith<sup>5</sup>) observed, that rabbits, when overloaded with carbohydrates, tolerated larger doses of phosphorus, than rabbits, kept on a mixed diet §

\*The most important of these substances is urea on account of its interaction with nitrous acid which is indispensable for the Millon test

†Fifty c.c. of the urine are mixed with 15 gm. of powdered  $Ba(OH)_2$  together with 1000 c.c. of a mixture containing two-thirds of alcohol and one-third of ether

‡For further details we refer to the above cited paper<sup>1</sup>

§So for instance a rabbit that had received 0.005 gm. of yellow phosphorus when fed with mixed food died within a week whereas another animal of nearly the same size that had received the double quantity of phosphorus and besides mixed food 30 gm. of glucose daily by the pharyngeal sound lived thirty days

The fact, that carbohydrates exhibit a protective power against phosphorus poisoning, was recently confirmed in this laboratory by R. Scholl's<sup>1</sup> experiments on rats. The minimal lethal dose of phosphorus for albino rats, when given subcutaneously, is 8 mg per kilogram body weight. The lethal dose is increased by a diet abundant in carbohydrates of 12 to 15 mg per kilogram. As carbohydrates starch and muhn<sup>2</sup> (yielding levulose on hydrolysis) were used. In those rats, which because of carbohydrate feeding had survived a lethal dose of phosphorus, the liver was found to be rich in glycogen and poor in fat, just the contrary was true for rats, which had succumbed to the intoxication. (See also Aindt and Giebling<sup>3</sup>)

It is generally known, that phosphorus causes a fatty degeneration of the liver, rather similar to acute yellow atrophy of the liver. Several clinicians<sup>4</sup> claim, that they were able to save cases of icterus gravis (beginning yellow atrophy of the liver) by the combination of intravenous sugar injections with insulin.

It is therefore quite logical, that different attempts were made, to treat cases by abundant administration of carbohydrates, where patients were affected with more or less severe icterus in consequence of an intoxication caused by an abuse of phenylcinchoninic drugs.

We therefore tried to establish by four series of experiments on cinchophen-poisoned rats, whether it is possible to influence this intoxication favorably by glycogen storage in the liver.

We came to the conclusion, that the *minimal lethal dose of cinchophen for albino rats was nearly the same, whether the diet was rich in carbohydrates or entirely free from carbohydrates*. These experiments again seem to prove, that in acute cinchophen intoxication a liver injury, provided that there is any, certainly does not play a dominating part. It goes without saying, that this does not prove anything for *delayed phenylcinchoninic intoxication in human beings* and that, in spite of the negative outcome of these experiments, it seems reasonable to try an abundant administration of carbohydrates in certain cases, where there is a suspicion of a beginning icterus gravis in consequence of an abuse of phenylcinchoninic drugs.

### III PHENYLCINCHONINIC INTOXICATION INFLUENCED BY HYPERTHYROIDISM

There are several indications, which suggest the idea that hyperthyroidism might possibly be one of those unknown factors, which, under certain conditions, in individuals uncommonly sensitive for phenylcinchoninic drugs, may cause a kind of "idiosyncrasy". It is generally known that in experimental hyperthyroidism as well as in exophthalmic goiter there is a greatly increased sensitiveness of the sympathetic nervous system. We know on the other hand, that phenylcinchoninic drugs exhibit an analgesic, antipyretic and antirheumatic action by influencing certain nervous centers, which are in close correlation with the sympathetic nervous system.

<sup>1</sup>It has been established by Neubauer that in phosphorus poisoning levulose is more readily stored in the liver than glucose.

The following experiments seem indeed to show that phenylcinchoninic intoxication in animals is markedly influenced by hyperthyroidism

a Eighteen *albino rats* were hyperthyroidized by thyroid feeding in the course of eight days. The normal lethal dose of cinchophen for rats is 0.55 to 0.65 gm per kilogram. It was diminished by hyperthyroidism (controlled by the progress of body weight) to 0.35 to 0.40 gm p.k.

b Three *guinea pigs* were hyperthyroidized in the course of sixteen days by 11 injections of thyroxin ( $\frac{1}{4}$  or  $\frac{1}{8}$  mg each). Doses of cinchophen of 0.4 gm per kilogram, corresponding to about  $\frac{2}{3}$  of the lethal dose (Furth and Kuh<sup>21</sup>) produced enormous falls of the rectal temperature to less than 34°.

c Three *rabbits* were hyperthyroidized by 6 injections of thyroxin ( $\frac{1}{4}$  or  $\frac{1}{2}$  mg thyroxin each) in the course of seven days. They died after having received 0.4 gm p.k. cinchophen subcutaneously, i.e. about one-third of the normal lethal dose.

We may learn from the foregoing experiments that the *toxicity of cinchophen is decidedly increased by simultaneous hyperthyroidism*.

#### IV. DIFFUSION OF DYE-STUFFS INTO GELATIN INFLUENCED BY CINCHOPHEN

The leading idea of our further investigations bearing on the mechanism of phenylcinchoninic intoxication was a *systematic study of colloidal changes induced by the phenylcinchoninic anion*. We began with the study of the diffusion of dyestuffs into gelatin as influenced by the presence of cinchophen.

K. Franke<sup>22</sup> at the medical clinic of Brugsch (Halle a. S.) observed an increased passage of eosin sodium from serum into gelatin in presence of a small quantity of atophan, and he considered this phenomenon as analogous to the increased passage of eosin into bile owing to the ingestion of atophan into the living organism.

a A hot 10 per cent solution of purest gelatin was poured into test tubes and allowed to solidify. A water solution of eosin 0.05 per cent, containing 0, 0.5, 1.0, 2.0 per cent of phenylcinchoninic acid (as sodium salt) was placed over the solidified jelly and the process of the diffusion of the dyestuff observed and measured.

EOSIN SOLUTION CONTAINING PHENYLCINCHONINIC ACID	THICKNESS OF THE DIFFUSION AFTER DAYS			
	1	2	3	4
per cent	measured in millimeters			
0	10	15	17	22
0.5	13	15	23	26
1.0	14	15	25	26
2.0	14	15	27	28

There was a well marked increase in the path of diffusion (*Diffusionsweg*) caused by the presence of the phenylcinchoninic anion.

b The experiment was repeated with eosin (0.05 per cent) and neutral red (0.05 per cent) and varied in such a way that the phenylcinchoninic anion was either contained in the color solution or in the solidified jelly itself. The reaction of the solution of cinchophen sodium was strictly controlled in view of its reaction and found to be practically neutral to litmus. The result was the same as in a.



c Diffusion of sodium sulphomdigoate (0.0125 per cent solution in water) into 10 per cent gelatin. The dyestuff solution (a) without addition, (b) containing cinchophen sodium 1 per cent, (c) containing sodium glycocholate 1 per cent, and (d) containing sodium salicylate 1 per cent. The glycocholate was used as a type of a substance exhibiting a high surface energy. The velocity of diffusion was markedly increased only by the presence of the phenylcinchoninic salt, but not by the salicylate, nor by the glycocholate. The path of diffusion (Diffusionsweg) measured after four days at room temperature was in (a) 10 mm, (b) 16½ mm, (c) 10½ mm, and (d) 11½ mm.

### CONCLUSION

Diffusion of certain dyestuffs (eosin, neutral-red, sodium sulphomdigoate) into gelatin is markedly increased by the presence of the phenylcinchoninic anion. This phenomenon does not seem to be due to changes in surface tension, but probably to colloidal changes in protein molecules.

### V SWELLING OF GELATIN INFLUENCED BY PHENYL CINCHONINIC ANION<sup>2</sup>

#### A

A 10 per cent gelatin solution was allowed to solidify in a crystallization dish. After cooling small cylinders of about 2 cm height were punched with a cork borer and weighed in small closed weighing bottles. Then the gelatin cylinders were allowed to stand for twenty hours in contact with either 0.9 per cent sodium chloride solution or with physiologic saline containing 10 per cent of sodium cinchoninate. The percentage increase in weight was established.

Physiologic saline as such	1.5919 gm 1.3991	1.8000 gm 1.2697	1.3001 gm 0.9481
	0.4923 +36%	0.5303 -28%	0.3520 -37%
Physiologic saline containing cinchophen	1.9964 1.2225	2.0674 1.3408	1.8003 1.1995
	0.6739 -58%	0.7266 -54%	0.6008 -51%

The percentage increase by swelling therefore was in the physiologic saline 36 to 38 per cent, in the physiologic saline containing in addition 10 per cent of cinchophen as sodium salt, 50 to 58 per cent, though the osmotic pressure of the latter, counteracting the swelling, was considerably increased by the presence of the salt or phenylcinchoninic acid.

#### B

In a further experiment we used a method, evolved by a Japanese author (Tomita<sup>3</sup>), which has the advantage of great simplicity. A hot 10 per cent gelatin solution is allowed to solidify in a crystallization dish of about 12 cm in diameter with flat bottom and cylindric walls. After cooling 30 cc of the fluid, which is to be examined, are poured in the crystallization dish, which then is closed by a cover in order to avoid evaporation. From time to time the fluid is poured off in a graduated cylinder and measured. The decrease in

<sup>2</sup> A. V. Kuthy (Biochem. Ztchr. 27: 296, 1921) found quite recently in the laboratory of E. A. Zbar (Debrecen) that those salts which belong to the type of hydrotropic substances (C. Neuberg), such as the sodium salts of benzoic acid, phenylacetic acid, phenyl propionic acid, benzyl-sulfonic acid, increase the swelling of gelatin and the passage of dyestuffs into gelatin. M. J. Popoff and K. S. Wilson (Veterinary School, Berlin, Biochem. Ztchr. 156: 196, 1925) already found that diethylene glycol increases the intake of water by gelatin.

its volume, indicating the intake of fluid by the swelling gelatin, is a relative index of the intensity of swelling. We used (a) NaCl 0.9 per cent and (b) NaCl 0.9 per cent, containing 1 per cent of the sodium salt of cinchophen. The intake of fluid by the gelatin was also in this case markedly increased by the presence of the phenyleinchoninic salt.

## C

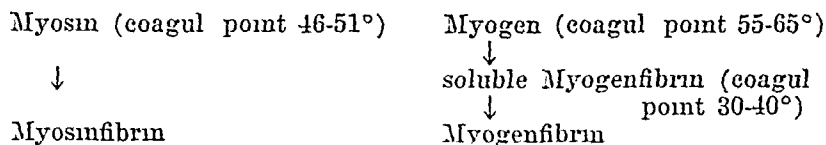
We performed several experiments in which, instead of gelatin, the swelling of frogs' muscles was studied. We used both the excised musculi gastrocnemii of a decapitated frog for each experiment and control, removing the adherent fluid carefully by hardened filter paper and weighing them accurately in closed wide mouthed weighing bottles. But we could not establish a marked difference caused by the presence of the phenyleinchoninic anion. This failure may be due to the gradual development of lactic acid from lactacidogen within the muscle. As lactic acid greatly increases the swelling of proteins, the effect of the phenyleinchoninic anion on swelling seems to have been masked by the superposition of this dominating factor.

## CONCLUSION

*The swelling of gelatin in 0.9 per cent sodium chloride solution is markedly increased by the presence of the phenyleinchoninic anion.*

## VI THE SPONTANEOUS COAGULATION OF TISSUE PROTEIN SOLUTIONS INCREASED BY PHENYLEINCHONINIC AND SALICYLIC ANION

More than thirty-five years ago one of us (Furth<sup>9</sup>) based on the earlier experiments of W. Kuhne (1864) and W. D. Halliburton (1888) studied in F. Hofmeister's laboratory the spontaneous coagulation of the muscle proteins myosin and myogen and he thought that it proceeds according to the scheme



In further researches, bearing on the interaction of certain poisons on the muscle proteins and their alleged influence on muscular rigidity<sup>10</sup> he observed, that some of these poisons greatly increased the spontaneous coagulation of myosin and myogen. The fact, that several of these drugs (sodium salicylate, antipyrin, quinine and cinchonin) are known as antipyretic and analgesic agents, gave us the idea to study the influence of the phenyleinchoninic anion on the spontaneous coagulation of tissue proteins.

*A. Muscle Plasma*—Muscle plasma was prepared by suspending ¼ kilo gram of hashed bovine muscles in ½ liter of physiologic saline and allowed to stand overnight. To samples of the filtered plasma were added equal parts of (a) NaCl 5 per cent, (b) cinchophen sodium 5 per cent and (c) sodium salicylate 5 per cent. (a) remained unchanged for more than one day and it was not before the second day, that a precipitate settled, (b) remained unchanged for one hour, after two hours there was a dense turbidity, which slowly after three days settled as a rich precipitate, (c) in the salicylate-containing samples there was a dense turbidity already after fifteen minutes and a rich precipitate settled in the course of the first day.

Myosin and Myogen were separated from the bulk of the muscle plasma, the first by addition of  $1\frac{1}{4}$  volume of saturated ammonium sulphate solution, the second by complete saturation with the same salt. The myosin and myogen precipitates were redissolved in water and the solutions of the proteins treated in the same manner as described for muscle plasma a, b, c. Myosin (a) remained clear for one day, (b) the cinchophen test began to become turbid after two hours, (c) contained an opaque turbidity already after one hour. Myogen (a) was unchanged after one hour and after one day there was only a poor precipitate, (b) and (c) settled to bulky precipitates already in the course of one hour.

There was no doubt, that the spontaneous coagulation of muscle plasma and its protein constituents is greatly increased by phenylcinchoninic acid.

**B Liver Plasma**—In quite the same manner a "plasma" was prepared from hashed calfs-liver. The result was analogous.

**C Minimal Effective Quantity of Cinchophen**—a Myogen solution, added with the equal bulk of cinchophen sodium solution, varying in their concentrations from 5 per cent down to 0.01 per cent. After five hours the samples, containing 5 to 0.2 per cent cinchophen, were decidedly turbid, the 0.1 to 0.01 per cent samples remained unaltered.

b Muscle plasma, comparison of the efficiency of the phenylcinchoninic and salicylic anion, dilutions varying between 5 and 0.01 per cent.

Cinchophen Na series. After one day the samples containing 15 to 0.3 per cent showed increased turbidity. The samples 5 to 2 per cent no turbidity. There was a reverse action, the precipitates being redissolved in an excess of the precipitant.

Sodium salicylate series. After one day there was a dense turbidity in the samples with 5 to  $1\frac{1}{2}$  per cent, slight turbidity in the samples with 1 per cent and 0.5 per cent. The samples with 0.4 to 0.01 per cent showed no more turbidity than the control samples without salicylate.

c Liver Plasma—Series of 15 samples, containing 25 to 0.005 per cent cinchophen sodium. There was an immediate, voluminous precipitation in the samples with 25 to 0.5 per cent. There was an immediate turbidity in the samples with 0.25 and 0.20 per cent. In the sample with 0.15 per cent cinchophen there was an opacity after half an hour. The series were placed in the incubator ( $40^{\circ}$ ) for three hours, whereas the control samples, containing no cinchophen, remained unchanged, there were decreasing precipitates in all the samples, even in those samples, which only contained 0.010 and 0.005 per cent of cinchophen.

**D Buffer Solution Series With the Sodium Salts of Phenylcinchoninic and Salicylic Acid**—It seemed to us important, to establish, how far the spontaneous coagulation of tissue proteins by the phenylcinchoninic and salicylic anion is influenced by the acidity of the medium. It is generally known, that the coagulation of proteins is increased mostly by increasing the acidity of the substrate. A series of phosphate buffer solutions was prepared according to P. Rona's<sup>11</sup> prescription by mixing m/15 solutions of  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  in adequate proportions. The  $\text{pH}$  of the resulting buffer solution was adequate to (alkaline end) 8.3 to 4.5 (acid end of series).

It became evident, that the spontaneous coagulation of muscle plasma is greatly increased by the presence of both the phenylcinchoninic and the salicylic anion and that this effect increases with the acidity of the medium.

**E Situation of the phenylcinchoninic and salicylic anion\* in the Hofmeister series**—It is known, that the efficiency of neutral salts in precipitating (salt-out) proteins is an additive function of their anions and cations, which,

\*The fact that salicylates stand beyond the rhodanid end of the Hofmeister series was discovered by R. Wilhelm (Kolloid Ztschr. 48: 270, 1929).

according to this quality, may be arranged into series (Holmeister's series) R Hober and other authors have shown that those anions which in an acid medium are the most efficient ones are the least efficient in an alkaline medium. Those anions, such as the iodide anion and the sulphocyanide anion which greatly increase the precipitation (dehydration) of proteins in an acid medium produce the contrary effect (increased swelling and solubility) in an alkaline medium. Holmeister's anionic series (the succession of anions in an acid medium is mostly but not quite uniformly registered as  $\text{CN}^- > \text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{Acetate} > \text{SO}_4^{2-} > \text{Tartrate} > \text{Citrate} > \text{F}^-$ ) is widely responsible for many physiologic and chemical phenomena in their dependence on the presence of certain anions. On account of the physiologic and pharmacologic importance of these facts it seemed interesting to ascertain, what place in the Holmeister series may be due to the phenylmchonnine and salicylic anions.

We may conclude from our experiments on muscle plasma and buffered solutions of muscle proteins that the phenylmchonnine as well as the salicylic anion are to be placed beyond the end of Holmeister's anionic series.

Phenylmchonnine anion }  $> \text{CN}^- > \text{I}^- > \text{NO}_3^-$   
Salicylic acid

*They increase the spontaneous coagulation (dehydration) of tissue proteins more intensively even than the sulphocyanide and iodide anion provided that the reaction is slightly acid.* The artificial solutions of tissue proteins are always slightly acid owing to the presence of lactic acid and they on the contrary will induce swelling (increased hydration) of tissue proteins provided that the reaction is neutral or slightly alkaline.

We therefore may possibly understand the mechanism of the lesion of liver cells by phenylmchonnine drugs from the point of view of colloidal chemistry. There may be increased swelling of the cells in the state of normal neutrality. No doubt that the increased swelling beyond a certain limit can injure the normal cell functions. It does not seem very probable that under the conditions of life the liver cells may ever become decidedly acid. But if owing to any pathologic incidence this might really happen the presence of the phenylmchonnine anion could possibly induce increased aggregation of protein particles, coagulation and necrosis.

#### VII INCREASED SPONTANEOUS COAGULATION OF PROTEIN SOLUTIONS FROM NERVOUS TISSUES BY PHENYLCHONINIC SALTS AND BY OTHER ANHYDRICS

The proteins of nervous tissues were first accurately studied by W. D. Halliburton.<sup>10</sup>

We prepared an extract from 250 gm. of finely minced cat's brain with 500 cc. of physiologic saline allowing the suspension to stand overnight and

\*For instance precipitation of proteins and of lecithin (swelling of gelatin and muscles, hemolysis, irritability of muscle and nerves, electric currents, arising from muscles, and nerves, intensity of ciliary motion, liberation of phosphoric acid from nucleicogen, etc.) (Buchhold,<sup>13</sup> Hober,<sup>12</sup> Embden and Lehnitz<sup>14</sup>).

†The dominating importance of the reaction of living tissues becomes evident from the experiments of Firth and Schwarz<sup>1</sup> on working musculi gastrocnemii of living cats left in situ. Whereas sodium salicylate and sodium sulphocyanide induce clotting of muscle plasma *extra corpus*, they greatly increase the energy production of the living working muscles.

centrifuging the strained turbid solution. This solution was saturated with ammonium sulphate, the precipitate of proteins collected on a folded filter paper, redissolved in about 20 cc of water and filtered again. Samples of  $\frac{1}{2}$  cc of the resulting clear yellow solution were transferred into small test tubes and added with the equal bulk of m/15 sodium benzoate, sodium salicylate, sodium salt of cinchophen, sodium benzoate of caffeine, hydrochlorate of quinine, antipyrine and m/10 solution of hydrochlorate of morphia. After half an hour precipitates had settled in the cinchophen and quinine<sup>2</sup> tests, and there was a slight turbidity in the salicylate test, the other tests were unchanged. The next day the benzoate, caffeine, and morphia tests were still unaltered, there were opaque turbidities in the salicylate test and antipyrine test. This experiment was repeated with the addition of phosphate buffer solutions.

We performed numerous series of experiments of the same type and came to the following conclusion. Six substances used as antipyretics, analgesics, and antirheumatics (phenylcinchonine and salicylic anion, quinine, antipyrine, phenocoll and melubrom) were found to accelerate the spontaneous coagulation of solutions of the proteins of nervous tissues, of the muscle and the liver. The first two agents appeared to be superior to the other substances in this respect. It will require numerous further experiments to show the efficiency or non-efficiency of other antipyretics and to establish definitely the sequence and order of their relative quantitative efficiency from this special point of view, which may of course be greatly altered by accidental factors and by slight changes in the true acidity of the solutions ( $P_H$ ). But the foregoing experiments seem sufficient for making it probable that some changes in the colloidal state of the proteins of certain nervous centers (increased or decreased swelling) may play a part in the pharmacologic and physiologic behavior of these and related substances. As the reaction in the living tissues is considered to be neutral or slightly alkaline, we should rather believe in an increased than in a decreased hydration, manifesting itself in the slightly acid medium of tissue extracts.

It was only recently that we learned to know the new important investigations of Bancroft<sup>3</sup> and his associates (Rutzel, Richter) at Cornell University on the relation of swelling of cerebral colloids to narcosis and the action of analgesic drugs. Sodium thiocyanate, which was chosen because it peptizes protein strongly and stands at the head of the lyotropic series of anions, was supposed to antagonize morphine. Sodium tartrate, which stands at the opposite side of the series, was claimed to synergize morphine. Overcoagulation of brain colloids was made responsible for manic depressive insanity, overpeptization for stuporous catatonia.

We hope soon to communicate further researches dealing with the interrelation between colloidal changes produced by analgesics and antipyretics and their toxicologic behavior.

\*As for quinine the pharmacologist Possbich<sup>4</sup> has observed more than half a century ago that the hydrochlorate of quinine depresses the coagulation temperature of egg albumen, muscle extract and blood serum. But no attention was paid to this discovery, the less so as the well known biochemist Miley<sup>5</sup> when reviewing this paper emphasized that the precipitation of proteins by the alkaloïds does not mean anything else than the withdrawal of alkali holding the proteins in a state of solution.

<sup>†</sup>As a water soluble substance closely related to pyrimidon.

## SUMMARY

1 A new functional liver test bearing an aromaturia (Furth and Scholl), was applied to the study of phenylelmchonnic intoxication in animals. This test is based on the separation of certain phenolic urinary substances, yielding the Millon reaction. In one case of subacute phenylelmchonnic poisoning and in 6 cases of subacute and acute intoxication in rabbits with the ethyl ester of p-methylphenylelmchonnic acid (Tolysin), there was no aromaturia, which if present would have indicated a severe injury of the liver. There only was aromaturia in a case of subacute phenylelmchonnic poisoning combined with hyperthyroidization.

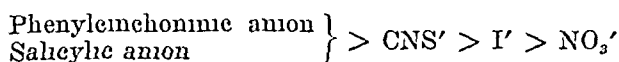
2 In view of the alleged possibility of detoxication of phenylelmchonnic acid by a diet abundant in carbohydrates, some experiments were performed in albino rats. The minimal lethal dose in acute intoxication was nearly the same, whether the diet was rich in carbohydrates or entirely free from them. (But the negative outcome of these experiments does not prove anything for delayed intoxication in human beings.)

3 The toxicity of phenylelmchonnic acid for albino rats, guinea pigs, and rabbits was markedly increased in association with hyperthyroidism. The features of phenylelmchonnic intoxication are dominated by the injury of certain nervous centers, which perhaps primarily may be due to colloidal changes.

4 This view is supported by the fact that the diffusion of certain dye-stuffs into gelatin is markedly increased by the presence of the phenylelmchonnic anion.

5 The swelling of gelatin in physiologic saline solution is markedly increased by the presence of phenylelmchonnic anion.

6 It seemed interesting to establish the position of the phenylelmchonnic anion in Hofmeister's anionic series, which are known to play an important part not only in colloidal chemistry, but also in many physiologic and pathologic phenomena. The phenylelmchonnic as well as the salicylic anion (R. Willhem) are to be placed beyond the end of the series.



These observations may possibly contribute to understand the fact, that an abuse of phenylelmchonnic drugs can induce in predisposed individuals colloidal changes in the liver cells, perhaps beginning as slight cloudy swelling and (in some exceptional and unfortunate cases) ending in a yellow atrophy of the liver.

7 Besides the phenylelmchonnic and salicylic anion several substances, used as antipyretics, analgesics, and antirheumatics (quinine, antipyrine, phenocoll, melubrin) proved to accelerate the spontaneous coagulation of protein solutions, resulting from brain, muscle, and liver. It seems probable, that *some changes in the colloidal state (degree of swelling) of the proteins, building up certain nervous centers*, may play a part in the physiologic and pharmacologic behavior of these and related substances.

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## A NOTE ON THE TREATMENT OF EXPERIMENTAL STREPTOCOCCUS MENINGITIS OF RABBITS WITH BACTERIOPHAGE\*†

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**T**HEORETICALLY at least the treatment of suppurative meningitis with bacteriophage would appear to offer a hopeful field for the clinical trial of this type of therapy, since it would appear possible to introduce sufficient amounts into the subarachnoid space to produce either lysis or the promotion of phagocytosis with special reference to the treatment of staphylococcus pneumococcus and streptococcus meningitis

We are indebted to Dr B F Stout of San Antonio, Texas, for suggesting this investigation on the possibilities of bacteriophage therapy in experimental meningitis and especially by subcutaneous and intravenous routes of administration, in view of his report on the recovery of a case of staphylococcus cavernous sinus thrombosis under this method of treatment<sup>1</sup>

Fortunately our colleague, Miss Clara Kast, was able to produce a virulent bacteriophage for our strain of hemolytic streptococcus employed for several years in the production of a rapidly fatal type of meningitis in rabbits by intracisternal inoculation. So far, however, we have not been able to produce a virulent phage for our strain of Type I pneumococcus likewise employed for

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several years in the production of pneumococcus meningitis of monkeys, dogs, and rabbits. Our experiments, therefore, have been confined to the bacteriophage treatment of experimental streptococcus meningitis of rabbits.

In these animals the resulting infection is very rapidly fatal and extremely difficult to control. For example, the intracisternal injection of 0.5 cc. of a 1:10 dilution of twenty-four-hour glucose brain hormone broth culture sometimes failed to produce meningitis altogether whereas the injection of a 1:5 dilution produced a severe purulent meningitis with septicemia ending fatally in eight to ten hours. As a general rule the rabbits were therefore inoculated with 1:6 to 1:8 dilutions and the resulting meningitis was usually fatal within twenty-four hours. In a few instances, however, untreated controls infected with these amounts of culture survived indefinitely, so that it was necessary to include a number of controls in each experiment and to exclude the results unless all untreated controls succumbed to the infection.

Treatment was started eight hours after intracisternal inoculation when the animals presented the severe classical signs of diffuse suppurative meningitis with purulent cerebrospinal fluids.

With some cisternal drainage was conducted about every twelve hours and the streptococcus bacteriophage injected intracisternally in dose of 0.5 to 0.75 cc. per rabbit (approximately 0.2 to 0.4 cc. per kilogram of weight). None survived and death usually occurred in forty-eight hours after treatment was instituted as shown in the results of an experiment summarized in Table I.

TABLE I

THE TREATMENT OF EXPERIMENTAL STREPTOCOCCUS MENINGITIS OF RABBITS WITH BACTERIOPHAGE

NUMBER OF RABBITS TREATED	PLAN OF TREATMENT	NUMBER OF DEATHS	SURVIVED	DIED	PERCENTAGE OF SURVIVALS
5	Cisternal drainage Bacteriophage intracisternally 0.5 to 0.75 cc. Bacteriophage intracarotidally 2.0 cc.	2	3	5	37
6	Cisternal drainage Bacteriophage intracisternally 0.5 to 0.75 cc.	3	0	6	0
6	Cisternal drainage Bacteriophage intracarotidally 2.0 cc.	2	2	4	33
16	Non controls	0	0	16	0

With others cisternal drainage only was conducted every twelve hours along with the injection of 1.0 cc. of bacteriophage into each common carotid artery corresponding to the very large dose of about 1.0 cc. per kilogram. With about 30 per cent of animals recovery followed the second or third injection and indicating that intracarotid injections were much more effective than intracisternal injections (see Table I).

Others still were given a cisternal drainage every twelve hours along with an intracisternal injection of bacteriophage in dose of 0.2 to 0.4 cc. per kilogram and an injection of approximately 1.0 cc. into each common carotid artery per kilogram. From 30 to 40 per cent of animals survived indefinitely (see Table I), so that this method of treatment gave the best results.



The results, therefore, are not without encouragement and particularly cisternal drainage combined with intracisternal and intracarotid injections of bacteriophage every twelve hours. So far the intracarotid injections have been most effective; unfortunately subcutaneous and intravenous injections have not yielded similar results in the few experiments conducted up to the present time. Smaller doses have likewise failed to yield similar results but this is largely a matter of concentration of virulent bacteriophage in the filtrates.

The time required and difficulties experienced in the preparation of autogenous streptococcus bacteriophage for the treatment of meningitis of human beings may militate against the application of this type of treatment in streptococcus meningitis of otitic or other origin but the results, so far, indicate the possibilities and merit serious consideration in the management of diffuse septic streptococcus meningitis in view of the extremely high mortality of this infection. As previously stated, we have not so far succeeded in producing sufficiently virulent bacteriophage for the different serologic types of pneumococci and no experiments have as yet been made with meningococci. The comparative ease of preparation of bacteriophage for staphylococci, however, greatly encourages the clinical trial of this type of therapy in staphylococcus meningitis.

#### SUMMARY

1 Cisternal drainage with intracisternal injections of bacteriophage have been ineffective in the treatment of severe rapidly fatal diffuse streptococcus meningitis with septicemia of rabbits.

2 Cisternal drainage along with intracarotid injections of bacteriophage have resulted in the recovery of about 30 per cent of animals.

3 Cisternal drainage with intracisternal and intracarotid injections of streptococcus bacteriophage have resulted in the recovery of 30 to 40 per cent of animals, and it would appear that this method of treatment is worthy of trial in the treatment of diffuse streptococcus meningitis of human beings.

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# SPONTANEOUS CARDIAC RUPTURE IN THE INSANE\*

## A REPORT OF SIX CASES†

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SIX cases of spontaneous rupture of the heart, occurring among 3257 deaths and 840 autopsies in a hospital for the insane, present an unusually high incidence of this dramatic fatality. Opportunity was taken for observations on associated changes, of which there is a dearth in much of the literature on this subject.

### REPORT OF CASES

**CASE 1**—J. L., a white man, aged sixty-four, had been an inmate of the hospital ten years because of alcoholic dementia, hypertension, and peripheral vascular sclerosis. There was muscular weakness with tremor and numbness of the right side of the face and the right arm and leg, enlargement of the heart with muffling of the sounds. The urine had a fixed low specific gravity and contained albumin. On November 22, 1927, while performing his usual light tasks in the hospital bakery, he walked across the room, reached upward for his coat and fell forward dead.

**Postmortem Examination**—The pericardial sac was completely distended with partially clotted blood. The heart (Fig. 1), weighing 590 gm., was elongated, firmly contracted, and infiltrated with subepicardial fat. On the anterior surface of the left ventricle, a linear ragged tear measuring 5 by 20 mm. roughly paralleled the long axis. It lay 30 mm. lateral to the anterior longitudinal sulcus and 35 mm. above the tip of the apex. In its center a small round opening admitted a probe directly and without resistance into the left ventricle. The inner aspect of the rupture appeared to the left and just below the origin of the anterior papillary muscle and the endocardium about it was covered with thin red thrombi. The cut surface of the myocardium was pale and striated with fine white markings. The left ventricular wall was uniform in thickness and measured 13 mm., the right, 8 mm. The valve rings were dilated. The chordae tendineae and tips of the papillary muscles were firm, thickened, and gray. The coronary arteries were rigid and atheromatous, and their lumina were irregularly diminished. The lower half of the anterior descending branch and the circumflex, as it turned right, was completely occluded by red thrombi. Other findings were chronic nephritis (together the kidneys weighed 100 gm.) and complete degeneration of the right cerebellar hemisphere.

**Histologic Examination**—The coronary arteriosclerosis was marked and the thrombosis was recent. A moderate perivascular fibrosis was observed in all the myocardial sections. There was extensive anemic infarction of the myocardium of the left ventricle involving the entire wall, at the site of the rupture and in the adjacent portion those muscle fibers lying next to the endocardium. There was no myocardial inflammatory exudate, no attempt at organization nor any pericarditis. The endocardium about the rupture was covered with fresh thrombi.

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†The cases used as the basis of this report including records of deaths and of autopsies are from the Warren State Hospital.

CASE 2—R S, a white man, aged seventy five, was in the hospital the third time because of a paranoid condition and arteriosclerosis. One morning (Feb 23, 1931) he was seized with excruciating abdominal pain followed by nausea and vomiting. During the afternoon the pain lessened somewhat. That night vomiting became more frequent and at 11 00 P M, he suddenly died.

*Postmortem Examination*—The pericardial sac was distended to its utmost capacity with partially clotted blood. The heart (Fig 2) weighed 410 gm, was elongated, flabby, and infiltrated with epicardial fat. A jagged tear 2 by 30 mm began at the tip of the apex and curved upward over the interior surface of the left ventricle near to and roughly parallel with the anterior longitudinal sulcus. In its center was a small opening which admitted a probe directly and without resistance into the left ventricle. Surrounding the tear was a globular aneurysmal dilatation measuring 3 by 40 by 70 mm which was studded with small hematomas. Viewed from within, the columnar carneae were stretched and flattened and the endocardium about the rupture was covered with thin red thrombi. There was infarction of the wall of the aneurysmal dilatation, of the adjacent posterior portion of the left ventricle and of the interventricular septum. The myocardium away from the rupture was mottled with coarse red and fine white streaks. The aortic ring was rigid and the mitral leaflets were covered with small, firm, translucent nodules. The distal two thirds of the anterior descending branch of the left coronary artery, and the terminal branches of the circumflex were completely occluded by red thrombi. There was marked arteriosclerosis of both coronaries which began near their origins. Measurements: left ventricular wall away from rupture 20 mm, right, 5 mm. The valves were all dilated. Generalized arteriosclerosis, anthracosis of the lungs, and passive congestion of the viscera were present.

*Microscopic Examination*—There was recent thrombosis of the blood vessels, fresh infarction of the wall of the cardiac aneurysm and adjacent muscle with cardiac endocardial thrombosis. Throughout the remainder of the myocardium there were interstitial and perivascular fibrosis and coronary arteriosclerosis. The pericardium was uninvolved.

CASE 3—E G, a white woman, aged sixty six, was admitted to the hospital Feb 14, 1931, because of a psychosis, senility, and neurosyphilis. The Kahn reaction on the blood serum was positive on two occasions. The heart was enlarged, the rhythm was irregular, and the blood pressure was 134/82. She became bedfast because of recurrent cardiac pain which radiated to the shoulders and was associated with circulatory collapse. She died suddenly in one of these attacks two weeks later (May 17, 1931).

*Postmortem Examination*—The pericardial sac was greatly distended with partially clotted blood. The heart (Fig 3) was short, wide, moderately infiltrated with fat and weighed 440 gm. A mottled infarcted, globular aneurysmal dilatation measuring 7 by 40 by 55 mm occupied the midportion of the posterolateral border of the left ventricle. In its center was a smooth, small, round hole, which exuded blood on pressure. With difficulty a small probe was passed obliquely downward into the left ventricle where it appeared between the origins of the papillary muscles. Covering the endocardium about its inner aspect was a thin, easily detached thrombus. There was coarse brownish red and fine white streaking of the myocardium. A few firm translucent nodules occupied the free margins of the mitral leaflets. Measurements: wall of left ventricle away from rupture 30 mm, right ventricle 7 mm. The valves were dilated. The chordae tendinae and tips of the papillary muscles were thickened, gray, and rigid. The openings of the coronary arteries were partially obstructed by hyalinized aortic plaques and puckered scars. The circumflex branch which ran directly into the infarcted area, the distal half of the left descending, and the right coronary artery just proximal to its bifurcation, were completely occluded by red and mottled thrombi, some of which could not be dislodged. There was marked generalized arteriosclerosis.

*Microscopic Examination*—The aorta showed mesaortitis. In places the perivascular infiltrations of round cells were dense and sufficiently necrotic to suggest miliary gummas. There were extensive areas of hyalinization and of calcification which in places occupied



Fig 1—Heart Case 1 A probe has been inserted into the rupture which has been sectioned

Fig 2—Heart Case 2 A section has been cut from the linear tear which lies near the apex. Note the aneurysmal dilatation about the rupture and the subepicardial hematomas

Fig 3—Heart Case 3 A probe has been inserted into the rupture which has been sectioned. Note the aneurysmal dilatation and the hematomas

Fig 4—Heart Case 4 A probe has been inserted into the rupture. Note the frayed appearance about the rupture and the aneurysmal dilatation which surrounds it

Fig 5—Heart Case 5 A probe has been inserted into the rupture. Note the extensive subendocardial hemorrhage

Fig 6—Heart Case 6 A dissection showing the course of the tear and above it the large subendocardial hemorrhage. On account of the tortuosity of the rupture the section was made partially tangential to the muscle fibers

four fifths of the media. Sections from the wall of the cardiac aneurysmal dilatation consisted of partially organized necrotic muscle fibers, red blood cells, granulocytes, polyblasts, and pigment. There were thin endocardial thrombi and chronic pericarditis. The coronary arteries were arteriosclerotic and some were occluded by thrombi recent and old. The smaller branches were infiltrated with lymphocytes and showed subintimal fibrosis. A section through the line of rupture consisted of free and hemolyzed blood which extended into the crevices between the friable and necrotic muscle fibers. Small infarcts in various stages of repair were scattered elsewhere in the myocardium.

**CASE 4—M. K.**, a white man, aged fifty-four, had been in hospital of the hospital half his life because of dementia praecox. Physically he was strong and vigorous and regularly did hard outdoor labor. He appeared to be in good health although a blood pressure reading of 178/106 was recorded. At 3:30 a.m., Dec. 19, 1931, he complained of thoracic pain which, because of language difficulties, was not clearly evaluated. Three hours later his face was unusually ruddy and his gait was slow and labored. At 8:00 a.m. he had a severe generalized convulsion accompanied by intense cyanosis. He died twenty minutes later without regaining consciousness.

**Postmortem Examination.**—The pericardial cavity contained 500 cc. of partially clotted blood. The heart (Fig. 4) weighed 350 gm. and was moderately infiltrated with fat. A linear frayed and ragged tear measuring 10 by 30 mm. occupied the lateral border of the left ventricle. It paralleled the long axis and admitted a probe without resistance directly into the chamber of the left ventricle. The heart wall surrounding the tear bulged to form a mottled brownish-red aneurysmal dilatation which measured 3 by 45 by 75 mm. The pericardium about this was covered with thin easily detached friable material with numerous hematomata beneath. The inner aspect of the rupture appeared just to the left of the base of the anterior papillary muscle and was surrounded by thin thrombi. The muscle of the apex was completely replaced by tough fibrous tissue which was found in lesser amounts throughout the myocardium. Both coronary arteries were atheromatous and the opening of the left was partially obstructed by an aortic plaque. Its branches were much thickened and their lumina diminished at many points. The circumflex branch which ran into the infarcted area was completely thrombosed 20 mm. from its origin. The left ventricular wall away from the dilatation measured 25 mm., the right, 7 mm. The mitral leaflets and base of the aortic cusps were thickened and rigid, and all the valves were dilated. There was generalized arteriosclerosis.

**Microscopic Examination.**—The changes in the aorta were characteristic of a mild mesoarteritis and a marked degenerative arteriosclerosis. The coronary arteries were arteriosclerotic, and many were completely occluded by thrombi. In a section through the aneurysmal dilatation, the pericardium showed chronic inflammation, the endocardium was covered with thrombi and the myocardium was infiltrated with polyblasts, granulocytes, lymphocytes, and fibrin in all stages of necrosis. Away from the infarct, interstitial myocardial fibrosis was extensive. No evidence of syphilis was found in other organs.

**CASE 5—J. S.**, a white woodsman, aged seventy-six, was referred from the county home, March 17, 1926, because of senility, hypomania and chronic alcoholism of long duration. There was peripheral vascular sclerosis, hypertension and muffling of the heart sounds. Urinalysis, blood chemistry, and the blood Wassermann and Kahn reactions disclosed nothing abnormal. He contracted a febrile upper respiratory tract infection, and two days later was seized with excruciating thoracic pain which radiated to the arm and which subsided sharply within an hour. Almost immediately it was followed by deep cyanosis of the left side of the face and chest, inaudible heart sounds, impalpable pulse, and unconsciousness. In a few minutes he was dead. Feb. 24, 1932. A diagnosis of coronary occlusion and infarction of the heart with probable rupture was made.

**Postmortem Examination.**—The pericardial sac contained 500 cc. of partially clotted blood. The heart (Fig. 5) was short and wide, moderately infiltrated with fat and weighed 500 gm. The visceral and parietal layers of the pericardium presented a buttered appearance. Both coronary arteries were tortuous, rigid and atheromatous, and the right was completely

thrombosed 20 mm from its origin. The entire posterior ventricular aspect of the heart presented a fatty mottling. An extensive subpericardial hematoma occupied the left auricular ventricular groove. Five millimeters to the left of the midportion of the posterior longitudinal sulcus was a smooth, round opening 2 mm in diameter. After fixation a probe inserted into this extended upward and inward 18 mm, and then turned sharply into the chamber of the left ventricle. Here it appeared just inferior to the right border of the mitral orifice, as a slit-like fracture in a columnar cartilage attached to the wall along one side. Below and to the left of it the interventricular septum was infarcted and bulged into the right ventricle to form a small aneurysmal dilatation. The endocardium about it was covered with thin thrombi. On section the myocardium immediately surrounding the rupture was soft and mottled brownish red (Fig 6), away from it there was fine white streaking. The interventricular septum and the line of insertion of the tricuspid valve was studded with subendocardial hematomas. The foramen ovale was patent, admitted a probe 4 mm in diameter, but was well guarded by a dense fibrous valve. The free margins of the mitral leaflets were thickened and nodular. All the valves were dilated. The wall of the right ventricle measured 7 mm, of the left ventricle at the site of rupture 8 mm, and away from the rupture 15 mm.

*Microscopic Examination*—The pericardium was covered with a fibrinous exudate which involved the areolar tissue about the base of the aorta. There was marked coronary arterio-sclerosis and the right was completely occluded by a partially organized thrombus. The myocardium about the rupture was converted into a partially organized infarct. Chronic myocarditis with perivascular fibrosis was present in other parts.

CASE 6—E S, a white woman, aged sixty-five years, was admitted to the hospital July 16, 1895, with chronic mania. Some years before death she had been edematous, partially paralyzed, and suffered a fracture of the femur. Dec 13, 1910, she was found dead in bed.

*Postmortem Examination*—The incomplete protocol recorded that the pericardial sac was filled with partially clotted blood. The heart was hypertrophied and dilated. There was a slit in the myocardium 25 mm in length which communicated with the right ventricle. Thrombi were found about the inner orifice of the rupture and also on the free margins of the tricuspid leaflets. About the base of the latter were calcific deposits.

#### COMMENT

Of these cases four were males and two were females. Their ages ranged between fifty-four and seventy-six years, the average being sixty-seven. The first five were encountered by one of us (Eaton) in a series of 140 routine autopsies performed at the Warren State Hospital between July 1, 1927 and Feb 23, 1932, during which period there were 691 deaths. In the records of 2566 deaths and approximately 700 autopsies between Jan 1, 1910 and July 1, 1927, only one such case was found. Since the files of the protocols are not entirely complete, other cases may have occurred. Combining these figures, there were six cases of spontaneous rupture in 840 autopsies and 3257 deaths. This is in keeping with the experience of Beersford and Earl of 31 cases in 2374 autopsies (Tooting Beck Hospital for mental and senile patients) and is sharply contrasted with that of others. Kumbhaan and Crowell 7 cases in 16,000 autopsies (Philadelphia General Hospital), Buckley 3 cases in 1330 autopsies (New Haven Hospital), and C. E. de la Chapelle 20 cases in 15,059 autopsies (Bellevue Hospital and Medical Examiner's Records, New York City).

Syphilis as a possible etiologic factor was noted in two cases. In Case 3 the evidence was indisputable. In Case 4 the partial obliteration about the origins of the coronary arteries and the histologic changes in the aorta were suggestive

although similar pictures have been described for other diseases (Pappenheimer and von Glahn, Moon and Stewart) The incidence of syphilis in spontaneous rupture varies from 45 per cent (Levine and Brown) to 50 per cent (Benson and Hunter) The latter group consisted of 14 ruptures out of 200 cases of fatal obstructive coronary artery disease, 17.5 per cent of which were syphilitic In certain cases of rupture where the patients are known to have had the disease, it appears to play a minor rôle (Stewart), and when not combined with arteriosclerosis, the cases fall into a younger age group (Krumbhaar and Crowell) The occupations of our cases seem not to have had any bearing although 1 and 5 were alcoholics

Cases 1, 4, and 5 had arterial hypertension, 2 and 3 did not Peripheral vascular thickening was marked in 1, 3 and 5, but was not looked for in the others All the patients were inmates of an institution for the insane The patient in Case 4 did hard labor, and the patient in Case 1, although the victim of a prior paralysis, did light work up to the day of death The others were decrepit and feeble In Case 1 death occurred after the patient had walked a short distance and reached upward The remaining five patients died in bed, two in an attack of acute cardiac insufficiency, one with and the other immediately following angina, one shortly after a convulsion which was preceded by angina, one after vomiting attacks which followed abdominal pain, and one during sleep In Cases 1 and 6 death was sudden without premonitory symptoms, Cases 2 and 4 were known to be "cardiac cases" In Cases 2, 3, and 4 coronary occlusion was suspected and in 5 positively diagnosed In our cases as in others (Longcope, Levine and Brown, Nathanson) and especially in the insane (Beesford and Earl), the clinical picture of coronary occlusion was not typical No symptoms pathognomonic of rupture were noted, although it is interesting that in 5, after the experience with four previous ones, the diagnosis of probable rupture was made An important sign of cardiac rupture is a sudden increase in the transverse area of cardiac dullness In all our cases the pericardial sac contained above 500 cc of fluid

It was not possible in any case to determine precisely the length of time which elapsed between rupture and death In traumatic ruptures where the coronaries and myocardium are not diseased, there is a definite interval In spontaneous cases, on the contrary, the diminished cardiac reserve is probably rapidly overcome by the added embarrassment to the already impaired circulation so that the interval, if one exists at all, must be short Krumbhaar and Crowell have presented histologic evidence to show that this is not always so Chapelle has reviewed the cases in which clinical evidence was presented to prove a definite interval Engelhart one case, twelve hours, Curtin one case, twenty-four hours, Osler one case, thirteen hours, Quain eight cases, one eight days, one six days, one three days, and five of them over forty-eight hours Chapelle believes it is probable that these were still in the stage of infarction Contributing to the cardiac failure is tamponade of the heart due to the rapid rise in the intrapericardial pressure, disturbances in cardiac rhythm, cerebral anemia, peripheral vascular arteriosclerosis and shock resulting from hemorrhage

The length of time lapsing between infarction and rupture is not so difficult to decide. Levine and Brown point out that the necrotic myocardium is softest between the fifth and fourteenth days. In three of our cases the infarct showed organization. In three others, judging from the histories and the postmortem appearances, rupture followed shortly after infarction.

In two cases the arteries occluded were the anterior descending and circumflex branches of the left coronary. In one case the circumflex branch alone, in another the right coronary and its branches, and in another (Case 3) all of them. In two cases the coronaries were partially occluded at their origins by changes in the wall. Cardiac aneurysm was noted in four cases, and myocardial fibrosis in five. Pericarditis and partial organization of the infarcts and thrombi were noted in 3 cases. In one case coronary thrombi of various ages were found which confirmed the clinical impression of repeated occlusions.

**The rupture.** In five cases this occurred through the left ventricular wall and in one through the right. Case 5 is remarkable in that the rupture did not take place into the right ventricular chamber for its general course was in that direction and only in abrupt change near the end brought it into the left. Of the first five, the tear was on the anterior surface near the apex in two, on the midportion of the lateral left border in two and near the midportion of the posterior longitudinal sulcus in one. These correspond to the common sites for cardiac infarct which Barnes and Ball have named respectively, the anterior apical, the midventricular and the posterior basal. In four the tear was linear, tortuous or curved and in two it consisted of a small round hole. In one this was partially covered by a thin flap. In all five a probe was admitted into the left ventricle. In two this passage was accomplished only after fixation and with some slight difficulty. The point is important for some ruptures are incomplete. In four the epicardial opening was larger than the endocardial while in one the reverse of this was true. In two the inner aspect of the rupture lay partially beneath the base and in two others between the origins of the papillary muscles, while in one it appeared in a split of a columnar cunea. The mechanism which actually brings about the rupture is usually thought to be a direct tearing of the muscle from the opposing pull of its own contraction or a giving way of the wall from pressure exerted upon it from within the chamber. The force of the former is much greater. Although the latter is the factor in certain cases as one which followed malignant endocarditis complicated by aortic stenosis and a high intraventricular pressure (Frankoff) which was somewhat similar to our last case. Important contributing factors are the loss of support ordinarily rendered by the excessive epicardial fat before it undergoes necrosis, thinning of the wall by an aneurysm, ulceration of the endocardial surface of the infarct, and separation of muscle fibers by capillary hemorrhage with the formation of multiple hematomas. Beresford and Earl consider that the blood caught among the muscle fibers is forced onward by contractions until a sinus tract is formed through the muscle and into the chamber. It seems of further importance to emphasize that in all our cases the mural thrombi, overlying the infarcted areas, were small and suggestively insufficient. In a previous case observed by one of us (Stewart), the endocardial surface of an extensive myocardial infarct was covered over by a thick thrombus except at the immediate



site of the inner aspect of the rupture which remained unprotected. The connection between fatty infiltration and spontaneous rupture has been stressed. Earlier pathologists considered this as the direct cause while recent writers tend to disregard it entirely. Beresford and Earl believe that an important relationship exists since disturbance in lipid metabolism is regarded as of etiologic importance in the senile psychoses. Davenport reports a high incidence of insanity in 92 recorded cases of spontaneous rupture. It is somewhat paradoxical that in those of our series in which rupture occurred through the left ventricular wall the subepicardial fat was largely confined to the right ventricle.

#### CONCLUSIONS

1. Six cases of spontaneous rupture of the heart occurring in patients who were inmates of an institution for the insane are presented and discussed.

2. In five coronary arteriosclerosis, coronary thrombosis and myocardial infarction preceded the rupture and in at least one of these syphilis was an etiologic factor.

3. In the insane the picture of coronary occlusion frequently is not typical.

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## V A DERMATOLOGIC TEST SUGGESTING AUTONOMIC IMBALANCE\*

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SINCE tissue extract contains "desympatone,"<sup>1, 2, 3, 4</sup> a hormone capable of neutralizing epinephrine, we thought that, like epinephrine, it might be utilized as a test for autonomic imbalance. However, we have not seen any comparable systemic response upon administering "desympatone" in therapeutic doses. We then directed observation to local reactions caused by this complex mixture as well as various fractions of this product.

We found that tissue extract, among many fractions, contains one, apparently a polypeptide, which produces a very interesting cutaneous reaction in many individuals. Because of its constricting local action, we have called this fraction "S" or "strictor" substance. It is not histamine, choline, or "desympatone." It is thermostable, is inactivated by alkalis and reactivated by acids.

It is prepared in the following manner:

Pancreas, kidney, or any other tissue is extracted with a hydroalcoholic menstruum containing hydrochloric acid. The extract is neutralized with ammonium hydroxide and filtered. The filtrate is acidified and concentrated in vacuo. Ammonium sulphate is added to the clear concentrate yielding a precipitate which contains the "S" constituent. This precipitate is extracted with alcohol, and any insulin removed if pancreas is used. The solution is concentrated in vacuo and passed through a Berkefeld filter leaving a residue which contains most of the "S" substance. This residue is dissolved in an acid menstruum to give a 3 per cent solution.

Different people and animals react differently to subepidermal injections of graded amounts of this substance.

The test is performed as follows. Of the solution, 0.025 c.c. is injected subepidermally (not intradermally) by means of a 26 gauge needle (Schick type) and a tuberculin syringe (Fig. 1). A bleb is produced (Fig. 2) (at times pseudopodia develop), and in the center a pearly white area appears (Fig. 3). Histologically we have found this to be due to focal tissue changes.<sup>†</sup> This usually develops in three to five minutes after injection, and is considered a "positive" reaction, providing it remains for more than twenty-four hours (Fig. 4). A "negative" reaction is one which fails to show this characteristic white center if 0.025 c.c. is injected (Fig. 5) or disappears in less than twenty-four hours. We

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have arbitrarily adopted this as our test dose. If a sufficiently large quantity is injected, a "positive" reaction may be obtained in most instances.

The more "S" substance required to produce this reaction, the more "negative" we consider the individual (Fig. 6).

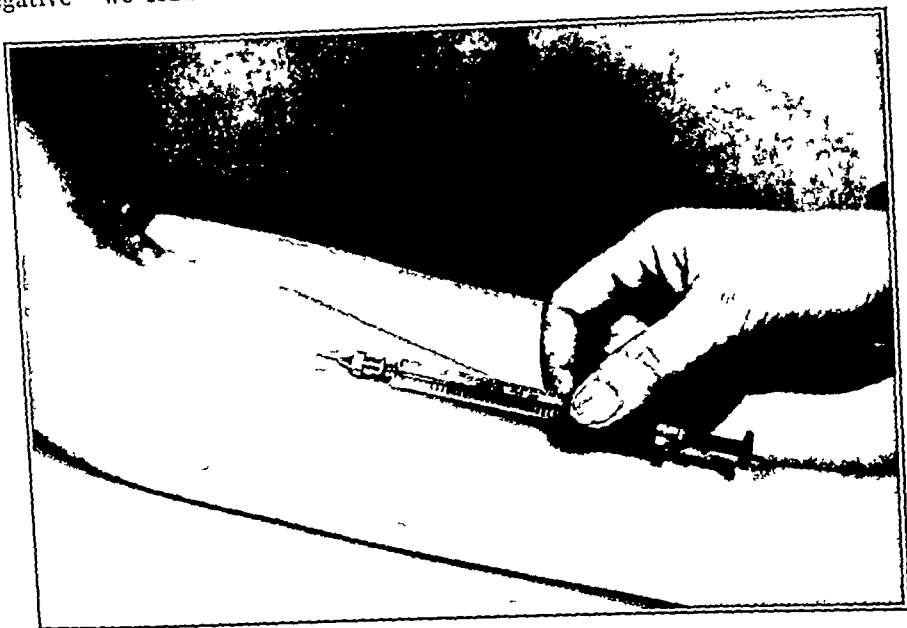


Fig. 1—Twenty-six gauge needle (Schick type) using a tuberculin syringe

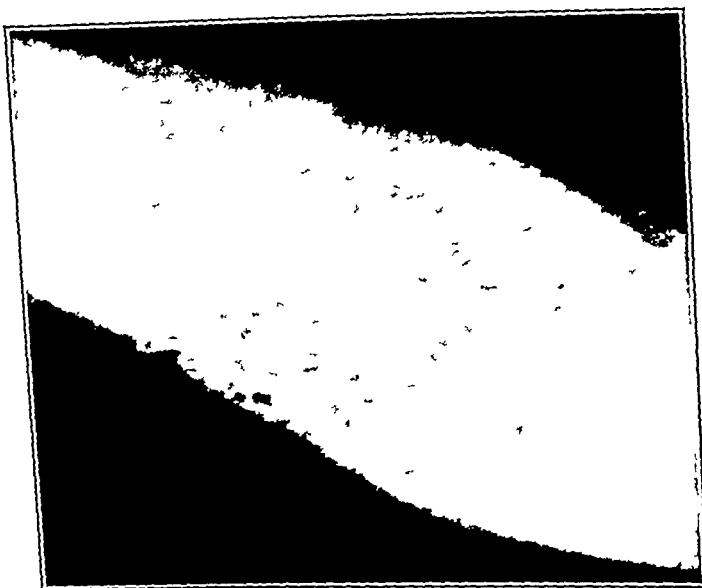


Fig. 2—A blch is produced with a central pearly white area just developing

If 0.025 cc fails to yield a "positive" reaction, the dose is gradually increased to 0.05 cc, 0.075 cc, 0.1 cc, etc, until a "positive" reaction is obtained.

After testing over 500 normal individuals and hospital patients, we were impressed with the fact that apparently the so called vagotonic, or para-sympatheticotonic individuals react characteristically to a small amount of "S" substance such as 0.025 cc. or even less.



Fig 3—Central area in a further state of development

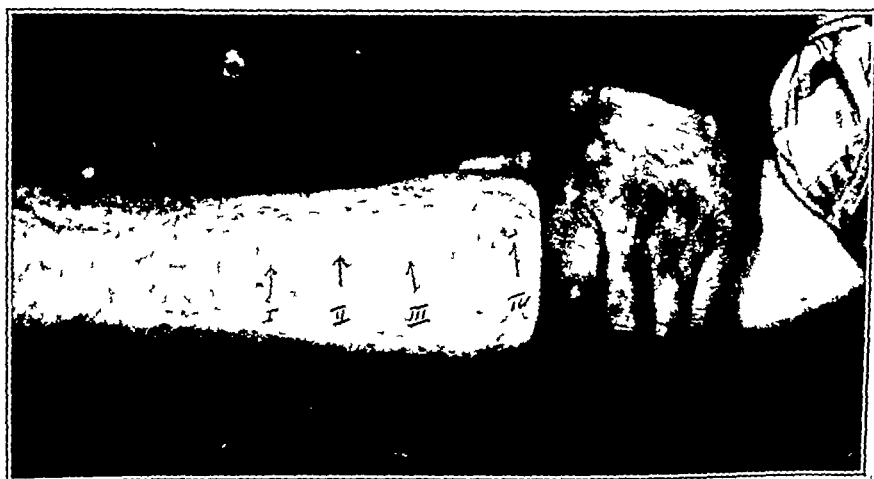


Fig 4—Twenty-four hours after injecting S solution. Response is shown to ascending amounts of the substance viz I 0.001 cc II 0.0005 cc III 0.01 cc IV 0.025 cc

So far, we have been unable to duplicate this reaction with substances such as milk, sera histamine and many others. The ease with which this test may be carried out and read may encourage others, particularly pharmacologists, to study it. More detailed findings will be published later.

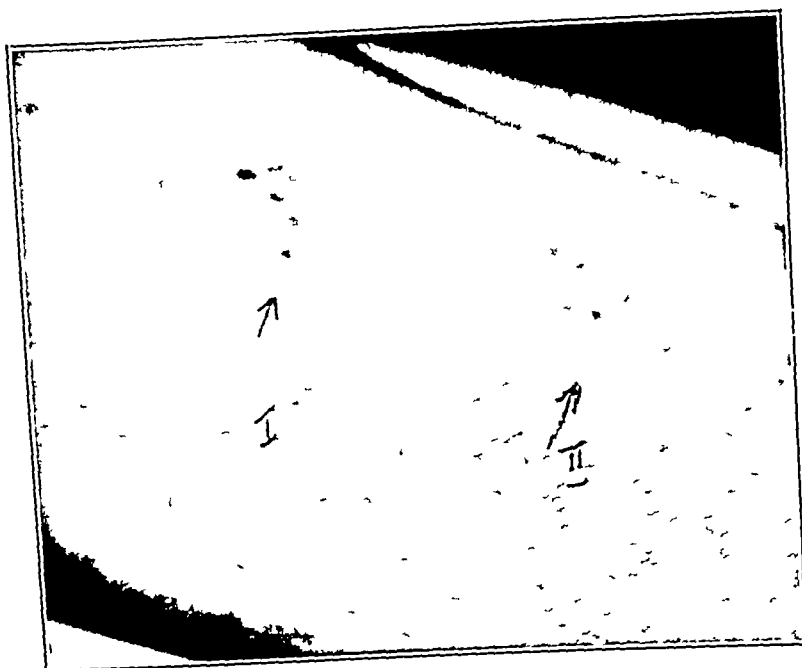


Fig 5—I immediately after injection II Five minutes after injection

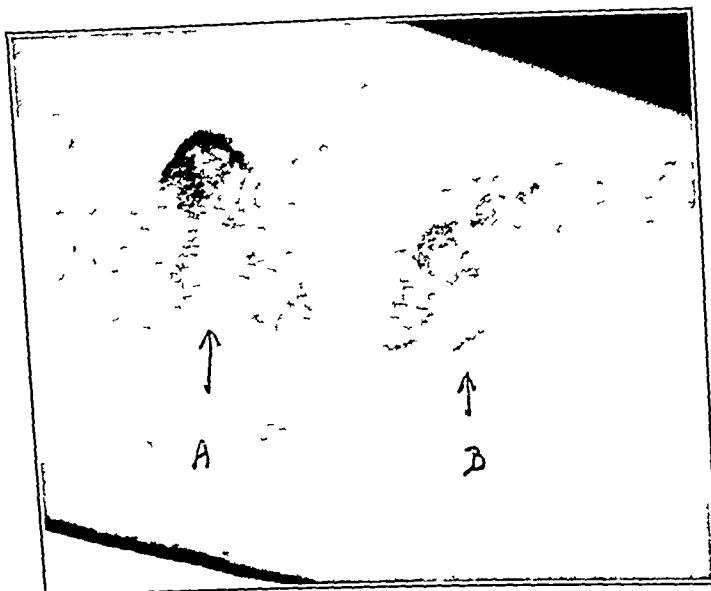


Fig 6.—This patient had jaundice and therefore the pseudopodia show up more definitely. A Shows a very minute response to 0.07 cc. B Shows a marked response to 0.2 cc. Light times is much substance as it would take a positive case to give a similar reaction

#### CONCLUSION

An S or stractor substance extracted from various tissues brings about different reactions in different individuals and animals. By a standard technique herein reported apparently some individuals have given a "positive"

reaction, while others have given 'negative' reactions. While suggestive that the reaction is influenced by the vegetative state, there is nothing conclusive to warrant its general adoption as a test until further researches are made.

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## AN EVALUATION OF THE EFFICACY OF OLEIC ACID WITH BILE SALTS IN ENTEROHEPATIC DISEASE\*

### A CLINICAL EXPERIMENT WITH TWENTY-FIVE SUBJECTS

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THE function and mechanism of the gall bladder and the manner of the emptying of bile into the duodenum are still the subject of much bewildering conjecture. It is an elusive and fascinating jungle trail which is being tickled persistently by a score of investigators in as many experimental laboratories.

Since the intimate relationship of hepatic to alimentary function has become established, the result of these investigations is increasingly significant because of its therapeutic import. Age or other physical disability forbids the benefit of surgical relief to one group of biliary tract sufferers. There are a considerable number of patients who for economic or other reasons will forego any medical assistance rather than agree to hospitalization or to bed management. Even with those who do consent to surgery, there is the well-known disturbing percentage of postoperative symptomatic recurrence.

The problem of the medical management of these cases becomes an imperative issue when one includes the large number of patients whose only symptoms are dyspeptic, for which no corresponding organic lesion can be demonstrated. Blackford and Dwyer<sup>1</sup> have found from their study of 1650 cases of chronic dyspepsia, that gall bladder disease occupies first place as a single cause of stomach complaint. They estimated that all organic stomach diseases together caused only 14.3 per cent of gastric symptoms, as against 20.4 per cent caused by derangement of the gall bladder. In their series, the approximate relative frequency of abdominal organic lesion causing dyspepsia was gastric ulcer, 1, gastric carcinoma, 2, reflex appendicitis, 4, duodenal

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ulcer, 6, *gall bladder disease*, 12 Dowling<sup>2</sup> in a later study of 1004 cases of gall bladder or bile duct disease found that the only early and prominent symptoms were dyspepsia in 64.5 per cent of these patients. It is these early dyspeptic patients who need the benefit of a rational medical procedure. It is logical to believe that such a therapeutic course is prophylactic against more serious involvement as well as alleviative.

Hence, there is an evident need for a medical mode of treatment which can be "proved up" in accordance with our newer experimental knowledge of biliary tract mechanism.

With this purpose in view, Lipschutz<sup>7</sup> in 1927 presented an experimental study of the efficacy of oleic acid with bile salts in emptying the human gall bladder and of stimulating a depleted bile flow, so as to evaluate the utility of such a medical treatment for relief of the biliary and gall bladder stasis which is a common factor in these cases.

In the present instance I have made a study of twenty-five subjects, using a different experimental approach so as to confirm or deny the previous findings. My results are the subject of this paper.

#### THEORETICAL BASIS OF THE EXPERIMENT

While it is not within the scope of this paper to review the divergent opinions which have been raised experimentally with regard to gall bladder mechanism, it will be explanatory of the theoretical basis of my series of experiments to recall certain measures of progress which have been made by various investigators with typically constant results.

To begin with, two momentous contributions have made it possible to evaluate human reactions more readily, which hitherto were not observable except by empirical conclusion. The first of these was the introduction of diagnostic (and therapeutic) nonsurgical biliary drainage by Lyon<sup>4</sup> in 1919, by which it is possible through use of the duodenal sound to observe the rate of discharge of bile into the bowel and the character of the biliary fluid. Lyon<sup>5</sup> has recently demonstrated further (1929) that good specimens of gall bladder bile may be obtained by duodenal tube after intraduodenal stimulation with certain substances and that in patients with unobstructed cystic ducts and less advanced cholecystitis, the gall bladder is the source of B bile and empties itself after certain intraduodenal stimulation.

The second contribution was the introduction by Graham, Cole and Copher<sup>6</sup> in 1924 of a method of visualization by the introduction into the body of dyes opaque to x-rays, which because of their concentration in the gall bladder make cholecystography practicable.

Various experiments, animal and human, have been reported to indicate the effect of foodstuffs and drugs upon both the secretion of bile and its emptying into the bowel. Of the types of food which encourage emptying of the gall bladder, it has been uniformly observed that fats are apparently unique. For the convenience of the reader, reference is made in the bibliography to some of the investigators who have arrived experimentally at this conclusion.<sup>11</sup> More recently, Babkin and Webster<sup>12</sup> in 1929, Higgins and Wilhelmj<sup>13</sup> in 1929

and Bassin and Whitaker<sup>14</sup> in 1930 have experimentally corroborated the earlier findings with regard to the dynamic influence of fats and fatty acids in promoting emptying of bile into the duodenum. Thus Bassin and Whitaker conclude that 'fats in emulsion given either by mouth or intravenously, appear to be the most constant and effective means of evacuating the gall bladder.'

It would, then, be logical but not practicable to feed the patient with fatty-rich meals, were they not so poorly tolerated. Administration of oleic acid is however, as extraordinarily effective in respect to evacuation of bile, and in addition eliminates the unavoidable disadvantages accompanying forced fat feeding. The efficacy of fatty acid as a gall bladder evacuant has been demonstrated by Copher and Kodama (1926),<sup>15</sup> Ivy and Oldberg (1928),<sup>16</sup> Stewart and Ryan (1928),<sup>17</sup> Ivy (1929),<sup>18</sup> and Randall (1931).<sup>19</sup> Thus Stewart and Ryan, making cholecystographic observations after administration of fourteen different drugs, found that the most constant response in the evacuation of bile from the gall bladder was obtained from oleic acid. They did not find that bile salts produced any evacuation.

Since there is usually a degree of diminution of bile secretion as well in the presence of enterohepatic stasis, therapeutically it appears rational to administer both oleic acid and bile salts whenever enterohepatic symptoms apparently due to biliary insufficiency indicate the advisability of medical treatment. Empirically and experimentally bile salts have long been known as powerfully choleric (promoting the secretion of bile, in contradistinction to cholagogue action on the evacuation of bile from the gall bladder). Recently the experimental choleric response after bile salts in dogs has been reported by Chabrol and Maxim<sup>20</sup> in 1929 and by Puestrow<sup>21</sup> in 1931. The former found that the secretion of bile tripled after the intravenous injection of bile salts, and the latter, that bile salts administered intravenously produced a profuse flow of thin yellow bile which lasted more than three hours."

#### EXPERIMENTAL

In the group of experiments conducted by Lipschutz,<sup>3</sup> he administered oleic acid in conjunction with bile salts\* to patients, and observed the efficacy of such a dosage in evacuating the gall bladder after administering the dye sodium tetraiodophenolphthalein salt and taking cholecystograms. The results were persistently uniform. Two hours after the ingestion of five capsules the cholecystograms invariably showed a diminution in the gall bladder shadow from one-half its original size to almost complete disappearance.

These experiments did not, however, afford a means of measuring any possible quantitative increase in bile secretion and the amount of bile evacuated into the duodenum. Thus I have endeavored to cover in my present experiment. In other words, I have tried to ascertain both the choleric and the cholagogue influence of such a dosage.

I used in my experiment 25 patients (20 females and 5 males). Sixteen were sufferers from chronic cholecystitis. Three others had peptic ulcer,<sup>4</sup> spastic colitis, and there were 2 normal controls.

\*Each soluble elastic capsule contained oleic acid 15 mins. sodium taurocholate <sup>1</sup>/<sub>4</sub> gr and sodium glycocholate <sup>1</sup>/<sub>4</sub> gr.



The purpose of the experiment was to ascertain by biliary drainage in each case over a two hour period the amount and kind of bile secured without stimulation after fasting, to ascertain on a second nonconsecutive day, the same data after stimulation with bile salts, and on a third nonconsecutive day, the same data after stimulation with oleic acid and bile salts in conjunction.

The technique on each day was as follows. After a fourteen hour fast overnight, the subject was prepared for duodenal drainage with a Jutte duodenal tube and placed in the Trendelenburg position so as to facilitate collection of the duodenal contents without loss. I have found this tube preferable because of the ease of passage of its small metal tip through the pylorus and its more ready facility in evacuating the duodenal contents through multiple perforations. During manipulation, the position of the tube was observed fluoroscopically. When it was in position, the duodenum was washed out with 10 cc of warm water administered through the tube and the tube clamped off. After ten minutes, the tube was unclamped and drainage of the duodenal contents continued for two hours, without suction and with each hourly quantity in the drainage collection bottle measured separately.

On the second nonconsecutive day of the experiment, the observations were made after the administration through the tube of 3 gr. of bile salts in 10 cc of warm water.

On the third nonconsecutive day of the experiment, the observations were made after duodenal administration of 6 cc of oleic acid in conjunction with 3 gr. of bile salts.

#### OBSERVATIONS

Table I gives the individual results.

1 Of the 25 subjects, 20 showed a greater outpouring of bile into the duodenum after bile salts than without stimulation of any kind. Of the 25, 24 showed a greater outpouring of bile after oleic acid with bile salts than without stimulation of any kind. The bile evacuation was considerably greater after oleic acid with bile salts than after bile salts alone, there being but one case where the former result was less than the latter.

2 B bile was not obtained in any case without stimulation or in any case after bile salts. It was typically obtainable after oleic acid with bile salts, being absent in only three cases. This corroborates the proposition of the dynamic effect of oleic acid upon gall bladder evacuation, if we are to consider as conclusive Lyon's observations that the gall bladder is the source of B bile. The average amount of B bile obtained was 24.32 cc. It was typically obtainable within five minutes after the stimulative influence was applied.

3 These experiments tended to show also that oleic acid is cholcretic as well as cholagogic, and that therefore it augments the secretory effect of bile salts upon the liver. One comes to this conclusion by comparing the quantity of bile (other than B or gall bladder bile) obtainable after oleic acid with bile salts, with the quantity of bile obtainable after bile salts alone. The amount of bile obtainable in the former case is typically a greater amount than in the latter case, there being but three exceptions in my series of 25 cases.

TABLE I  
AMOUNT OF DUODENAL BILE PROCURED

CASE	WITH NO STIMULATION	AFTER BILE SALTS STIMULATION	AFTER OLEIC ACID AND BILE SALTS STIMULATION		
			AMT BILE OTHER THAN B BILE	AMT B BILE	TOTAL
1	35 cc	40 cc	75 cc	15 cc	90 cc
2	53 cc	68 cc	165 cc	0 cc	165 cc
3	17 cc	25 cc	40 cc	10 cc	50 cc
4	80 cc	92 cc	120 cc	22 cc	142 cc
5	92 cc	80 cc	75 cc	8 cc	83 cc
6	40 cc	55 cc	73 cc	12 cc	85 cc
7	95 cc	115 cc	240 cc	23 cc	263 cc
8	102 cc	118 cc	172 cc	15 cc	187 cc
9	112 cc	105 cc	100 cc	25 cc	125 cc
10	29 cc	25 cc	42 cc	10 cc	52 cc
11	0 cc	12 cc	33 cc	12 cc	45 cc
12	0 cc	31 cc	25 cc	0 cc	25 cc
13	123 cc	135 cc	285 cc	100 cc	385 cc
14	111 cc	128 cc	240 cc	75 cc	315 cc
15	87 cc	95 cc	137 cc	32 cc	169 cc
16	12 cc	0 cc	132 cc	0 cc	132 cc
17	180 cc	240 cc	315 cc	45 cc	360 cc
18	144 cc	152 cc	211 cc	35 cc	246 cc
19	300 cc	322 cc	387 cc	27 cc	414 cc
20	172 cc	210 cc	245 cc	32 cc	277 cc
21	93 cc	111 cc	178 cc	22 cc	200 cc
22	242 cc	264 cc	341 cc	17 cc	358 cc
23	42 cc	73 cc	192 cc	13 cc	205 cc
24	143 cc	128 cc	183 cc	21 cc	204 cc
25	278 cc	294 cc	350 cc	37 cc	387 cc
Total	2582 cc	2918 cc	4356 cc	608 cc	4964 cc
Average	103.28 cc	116.72 cc	174.24 cc	24.32 cc	198.56 cc
Average increase %		13%			92.2%

4 With regard to percentage of increase in amount of bile obtainable after stimulation, in the case of stimulation with bile salts, omitting 4 anomalous cases, the lowest increase was 5.5 per cent, the highest, 73.8 per cent, and the average increase of all 25 cases, 13 per cent. In the case of stimulation with oleic acid in conjunction with bile salts, omitting 4 anomalous cases, the lowest increase was 11.6 per cent, the highest, 388 per cent, and the average increase, 92.2 per cent.

5 Of the atypical reactions, the following were observed. In Case 5, both bile salts alone and oleic acid with bile salts failed to produce as much duodenal bile as was obtained without stimulation. In 4 other cases (9, 10, 16, and 24), bile salts alone failed to produce bile increase, although oleic acid with bile salts effected some increase. In one anomalous case (12) oleic acid with bile salts produced less bile increase than did bile salts alone, and produced no B bile. There were two other instances in which B bile was not procured after oleic acid with bile salts.

6 Table II presents percentages of increase, and Chart 1 compares graphically the average bile measurements procured in the three different phases of my experiment.

7 I realize that there are controversial views with regard to the accuracy of duodenal drainage as a method of recovering all of the bile from the duodenum. A point in favor of its accuracy is the uniformity of the results I secured in my 25 experimental cases. It is the opinion of some investigators<sup>22</sup> that this method of drainage procures from 94 to 99 per cent of duodenal increment.

▨ B bile      ■ other than B bile

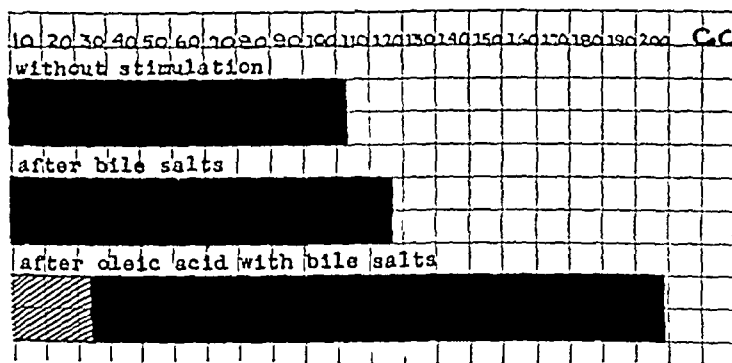


Chart 1—Average cubic centimeters of bile recovered from duodenum in twenty-five subjects.

TABLE II

INCREASE OF BILE AFTER STIMULATION OVER AMOUNT SECURED WITHOUT STIMULATION

CASE	AFTER BILE SALTS	AFTER OLEIC ACID WITH BILE SALTS
1	14.3%	157.1%
2	28.3%	211.3%
3	47.0%	194.1%
4	15.0%	77.5%
5	-----*	-----†
6	37.5%	112.5%
7	21.0%	176.8%
8	15.6%	83.3%
9	-----	11.6% low
10	-----	79.3%
11	Approximately 1200%*	Approximately 4500%†
12	Approximately 3100%*	Approximately 2500%†
13	9.7%	213.0%
14	15.3%	183.7%
15	9.2%	94.2%
16	-----*	1000.0%†
17	33.3%	100.0%
18	5.5% low	70.8%
19	7.3%	38.0%
20	22.0%	61.0%
21	19.3%	115.0%
22	9.0%	47.9%
23	73.8% high	388.0% high
24	-----	42.6%
25	5.7%	39.2%

Average percentage of increase

After bile salts ----- 13.0%

After oleic acid with bile salts ----- 92.2%

(figured by averaging totals of cc in Table I)

\*Cases considered anomalous. Omitting these anomalous cases the lowest percentage of increase was 5.5% the highest, 73.8%.

†Cases considered anomalous. Omitting these anomalous cases the lowest percentage of increase was 11.6% the highest 388%.

My experiment has corroborated the observations of Lipschutz and Levinson,<sup>2,3,4</sup> who also experimentally and clinically found that administration of oleic acid with bile salts is an effective means of relieving biliary stasis.

#### SUMMARY AND CONCLUSIONS

The bile procurable by duodenal drainage was measured as follows in 25 subjects: on the first day, without duodenal stimulation; on the second non-consecutive day after duodenal administration of bile salts; and on the third day, after similar stimulation with oleic acid and bile salts in conjunction.

There was a typical increase in bile flow after bile salts, and also a typical and much greater increase after oleic acid with bile salts. In the former instance, the average percentage of increase was 13 per cent, in the latter, 92.2 per cent.

Bile (gall bladder bile) was procured only after stimulation with oleic acid and bile salts in conjunction.

Barring the inaccuracies due to unknown factors, the experiment demonstrated that oleic acid is strongly dynamic as a cholagogue (evacuating the gall bladder) and also is definitely dynamic as a choleretic (increasing bile secretion), and that bile salts are dynamic as a choleretic and not at all as a cholagogue.

Therapeutically it suggested that the experiment demonstrates the preferable efficacy of administering oleic acid and bile salts conjointly whenever medical treatment is in order in enterohepatic disease accompanied apparently by biliary stasis.

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57 WEST FIFTY SEVENTH STREET

## CARCINOMA OF THE STOMACH WITH METASTASES TO THE SKIN\*

### CASE REPORT

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GENERALIZED metastases to the skin from carcinoma of the stomach is not a usual occurrence. A careful search of both the domestic and foreign literature reveals not more than 137 such cases. Nobuyoshi Suzuki<sup>1</sup> in commenting upon the rarity of this condition states that Richelman reviewed a series of 711 cases of gastric cancer, and Gussenbauer and Winiwarter studied 903 such cases and failed to find a single case presenting secondary tumors to the skin. Lubarsch, quoted by Guilbert,<sup>2</sup> found only 0.4 per cent of a series of 2738 cases of gastric cancer metastasizing to the skin. When such metastases occur, they are found more commonly in the region of the umbilicus. In only a few of the reported cases were the nodules very numerous. Of all the abdominal cancers metastasizing to the skin, those of gastric origin, according to Bonquetot,<sup>3</sup> hold the first place while intestinal and uterine cancers come next in frequency. Complete case reports have been presented notably by Croizon,<sup>4</sup> Loeper and Turpin,<sup>5</sup> Menetrier and Gaukler,<sup>6</sup> Merklen,<sup>7</sup> Maunoury,<sup>8</sup> Duijsbeek,<sup>9</sup> Suzuki,<sup>10</sup> and Uhlenbruch and Gilardone.<sup>11</sup>

The following case report is presented including complete necropsy findings.

### CASE REPORT

*History*—The patient, a colored female, aged forty-two, was admitted to the medical service of the Montefiore Hospital on October 24, 1932 complaining of marked dyspnea and pain in the abdomen. She felt perfectly well until three months previous to her admission when she began to be troubled with a slow dull, aching pain in the upper abdomen, this

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pain radiating to the small of the back. It usually occurred about one hour after meals, and was relieved chiefly by vomiting. Her appetite was very poor. There was no history of hematemesis or blood in the stools. Her bowels moved only following catharsis. About the time of the onset of the above symptoms, she noticed a gradual but painless enlargement of her abdomen. One month previous to her admission she began to notice variable sized masses occurring in the skin over the anterior surface of the chest and abdomen. During this interval she lost about fifty pounds in weight. For a few months she had also been troubled with increasing shortness of breath on exertion. There was no edema of the ankles, no cough, and no hemoptysis. She presented no symptoms referable to the respiratory or urinary systems. Her menstrual periods were irregular. She had been married for thirteen years but was never pregnant. The past medical history included measles, mumps, pneumonia, typhoid fever, and influenza. There was no history of venereal diseases, repeated sore throats, skin rash, or miscarriages. Her father was living and well. Her mother died at forty five due to childbirth. There was no history of tuberculosis or cancer in the family.

*Physical Examination*—The patient was an adult, colored female, weighing about 110 pounds. Temperature 98°, pulse 120, respirations 22, blood pressure 140/80. On admission she appeared emaciated, pale, apparently acutely ill, and suffering from a great deal of pain. The skin over the entire chest and abdomen, anteriorly and posteriorly, was covered



Fig 1—Anterior view showing metastasis to skin

with numerous irregular, hard, subcutaneous nodules varying in size from a pea to a walnut (Fig 1). Some of these nodules were freely movable beneath the skin, and others were more firmly attached. Over several of these the skin was atrophic and showed signs of chronic irritation with a tendency to ulceration. There was one walnut sized tumor over the left malar process, and several over the right thigh. Supraclavicular nodes were not palpable. The pupils were unequal, the left being larger than the right, and the right was definitely irregular. Reaction to light was sluggish but both pupils reacted to accommodation. Posterior synechia were present. On the right side there was moderate retinal arteriosclerosis. Otherwise the fundi were negative. The ears and nose were negative. The mouth was dentitionless. The tonsils appeared imbedded and slightly cryptic. The submaxillary lymph nodes were enlarged and palpable. Heart and lungs were negative. The abdomen showed definite enlargement which was apparently due to a massive involvement of the liver so that the edge of the liver extended about 3 cm below the umbilicus. The organ appeared hard, firm, and suggestively nodular on palpation. The left kidney region presented some tenderness. The spleen was not palpable. The knee jerks were equal but decreased. No pathologic reflexes were present. Pelvic examination revealed the presence of infantile pelvic organs and a retroverted and adherent uterus.

*Laboratory Examination*—Urine was negative. Phenolsulphonephthalein, first specimen, 12.2 per cent, second specimen, 9.5 per cent, total 21.7 per cent. Blood chemistry sugar,

93 mg, nonprotein nitrogen, 31 mg. Several blood counts performed during the patient's stay in the hospital showed a progressive anemia, thus, on Oct 26, 1931 hemoglobin, 60 per cent, RBC, 3,400,000, WBC, 8000, differential, normal. On Nov 18, 1931 hemoglobin, 25 per cent, RBC, 1,650,000, WBC, 8000, differential, normal. Wassermann and Kahn tests were negative. Following a provocative test the blood Wassermann became positive and remained positive throughout the patient's stay in the hospital. Stool examinations were negative for blood and parasites. Fractional gastric analysis was negative for

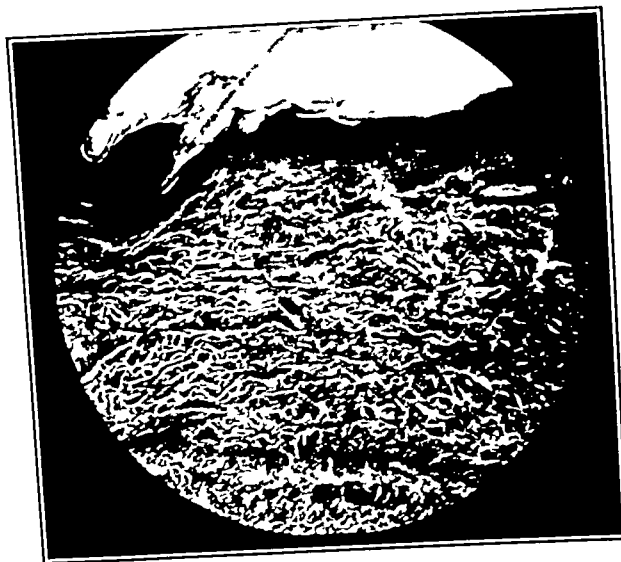


Fig 2—Section of skin nodule (low power) showing carcinomatous infiltration.



Fig 3—Section of skin nodule (high power)

lactic acid, but positive for blood, the specimen showed achlorhydria and a low combined and total acidity. Gastrointestinal roentgenographic study showed a constant filling defect at the pyloric end of the stomach. The character of this defect was suggestive of an annular carcinoma of the pars pylorica. X ray study of the colon by aid of barium enema revealed a slight narrowing of the descending colon about five inches from the rectosigmoidal junction. There was some ptosis of the colon but no evidence of any permanent obstruction. A roentgenogram of the left maxillary bone taken from the region of the subcutaneous nodule failed to reveal any metastases to the bone. Biopsy was made of one of the skin nodules

Grossly the specimen submitted consisted of two irregular masses covered with adipose tissue. On section they were firm and consisted of a pearly gray tissue infiltrating the surrounding structure. Microscopically the structure was composed of epithelial like cells which infiltrated the stroma in all directions. The nuclei were hyperchromatic, and numerous mitotic figures were seen. The stroma was dense connective tissue. Scattered through the tumor were areas of degeneration and lymphocytic infiltration. Generally, the picture was that of carcinoma with degeneration (Figs 2 and 3).

*Diagnosis*—Carcinoma of the stomach with generalized metastases to the skin and liver. Syphilis. Secondary anemia.

*Course*—The patient was given symptomatic treatment. In spite of the positive serology it was felt that syphilis did not play an important role in the existing clinical condition. Therefore, because of this consideration as well as because the patient was so acutely ill, antisyphilitic treatment was not forced. The course of her condition was uneventful until November 11, or about one month following her admission, when she suddenly developed a severe gastric hemorrhage, vomiting bright red blood. She recovered sufficiently from this hemorrhage and got along fairly well until January 3, when she complained of a sudden abdominal pain after which she became pulseless, showed all the signs and symptoms of severe shock, and then died in two hours.

*Necropsy*—The body was that of an adult colored female showing marked emaciation. The skin of the chest wall, anteriorly and posteriorly was studded with firm round tumor masses, some of them fixed and others movable. The skin covering the tumor masses was intact, except in two places where ulceration was seen. The abdominal wall had similar nodules, but fewer in number. The extremities showed only two similar nodules, in the left thigh. There was a large lump the size of an orange in the left zygomatic region, fixed to the bone. The eyes, ears, and nose were grossly negative. The primary incision passed through a tumor mass on the chest wall. The cut surface of the tumor showed central necrosis. The peritoneal cavity contained about 2 liters of brownish red slightly turbid fluid. The parietal peritoneum was studded with numerous nodules. The abdominal viscera were found in their normal position and relation, but adherent to each other, with old and new adhesions partly due to neoplastic involvement, and partly to recent peritonitis. Thorax. The lungs were semicollapsed. The right pleural cavity contained about 300 cc of serous fluid. The left cavity was free of fluid. There were numerous fine old adhesions between the layers of the pleura. The pericardial sac contained the usual quantity of straw colored fluid. Both lungs were identical in gross pathology. They had the usual anthracosis of the district and several foci of healed lesions of tuberculosis and moderate degree of terminal edema. No macroscopic evidence of neoplastic involvement was found. The peribronchial lymph nodes showed slight enlargement and discoloration. One was involved with tumor growth. The heart was rather small, and the pericardial covering was smooth. The myocardium was pale and flabby. The endocardial lining was smooth. The aortic valve showed moderate thickening and atheromatous changes. The other valves were normal. The arch of the aorta was moderately dilated and the intima showed longitudinal striations. The opening of the coronary vessel was patent, and the vessels were free from atheromatous changes. Abdomen. The liver was markedly enlarged and distorted. It weighed 32 hg. The right lobe was extensively involved with tumor growth. The surface of the liver was rough and nodular. More than half of the liver parenchyma was replaced by tumor masses. These were pearly gray, very firm in consistency, with a tendency to central necrosis. The left lobe of the liver was free from such involvement. The gall bladder and the large biliary ducts were normal. The ampulla of Vater was patent. The pancreas was normal. The tail of the pancreas was adherent to the spleen and to the upper pole of the left kidney. The spleen was moderately hyperplastic. The capsule was thick and was infiltrated with several tumor nodules. The splenic tissue proper showed moderate degree of congestion and softening, but no involvement by tumor. The left kidney showed nodules at the upper pole. The rest of the organ was pale. No definite line of demarcation was evident between the cortex and the medulla. The right kidney was free from metastasis. The internal genitalia



were essentially negative except for a few old adhesions. The ovaries were atrophic, and no neoplastic changes were present. **Gastrointestinal Tract.** The stomach was collapsed. There was a perforation in the lesser curvature, about 4 cm from the pylorus on its interior aspect. On opening the stomach, there was a firm tumor mass the size of a silver half dollar. This mass showed ulceration in the center, with a perforation measuring 2 mm in diameter. The lymph nodes both at the lesser and greater curvatures showed metastatic involvement. The mesenteric lymph nodes were generally involved. The lumen of the intestines were free.

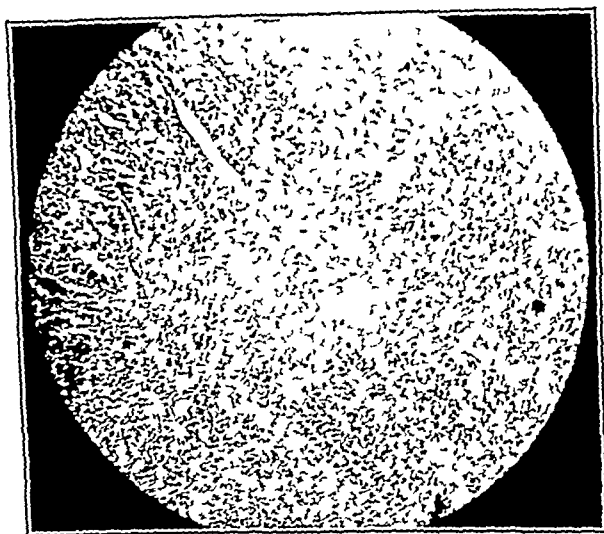


Fig 4—Section of lesion in stomach (low power) showing carcinomatous degeneration

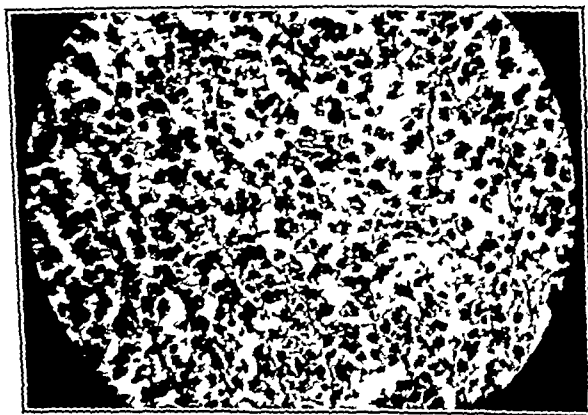


Fig 5—Section of carcinoma of the stomach (high power)

from involvement, except the lower segment of the sigmoid flexure where there was a large nodular mass infiltrating the wall but not obliterating the lumen.

**Microscopic Studies.**—Sections taken from various tumor masses showed similar changes. There was a marked anaplasia of the cells. The cells were epithelial in type with mitotic figures and hyperchromatic nuclei. There were areas also with extreme degeneration where it was impossible to make any differentiation of nuclear structures. They were faintly stained with eosin. The original tumor mass from the stomach (Figs 4 and 5) did not show any tendency to acinar formation, but the tumor cells showed typical invasive characters.

extending through the stroma which was dense fibrous tissue. Sections taken from the liver, kidney, and the rest of the organs showed areas of tumor cells, marked leucocytic infiltration, and occasional giant cell structure resembling that of foreign body giant cells. Sections of the spleen, lung, and pancreas did not show any evidence of metastatic involvement.

*Anatomic Diagnosis*—Scirrhus carcinoma of the stomach with ulceration and perforation. Generalized peritonitis. Intraabdominal hemorrhage. Generalized metastatic carcinoma of the liver, kidney, lymphatics of omentum, and mesentery. Massive metastasis to the skin. Terminal edema of lungs.

#### COMMENT

Syphilis was present in several of the cases reported in the literature. In some of these cases it was thought at first that the nodules were of syphilitic origin. The biopsy findings, however, ruled with certainty against such an assumption in the case presented above.

The case was somewhat unusual because it presented no involvement of the lungs which are, as a rule, included in such metastatic processes. Furthermore, when skin metastases occur they usually are associated with adenocarcinoma of the stomach rather than with the scirrhus type of cancer.

With regard to the route of distribution of the cancer cells to the skin there are two views—the hematogenous and the lymphogenous. In favor of the hematogenous distribution of these metastases is the fact that the metastases involve various viscera, the pericardium, the suprarenal glands as well as the skin, and, furthermore, that they are frequently not associated with any regional adenopathy. A biopsy of the skin lesions frequently shows the presence of many blood vessels which are surrounded by cancer cells. The nodules may appear simultaneously. There may be no dilatation of the lymph vessels and lymph spaces in the skin, and there may be cancer cells in the blood vessels and capillaries of the metastatic skin nodule, as well as in the blood vessels of other organs involved in the malignant process. On the other hand, Vedel, Puech, and Chardonneau<sup>12</sup> take the opposite view. Likewise Guilbert<sup>2</sup> has never noticed a lesion involving the arterial wall or cancerous emboli to the interior of these vessels. In some of the sections studied by him he noticed the lymphatic vessels in the skin to be distended with neoplastic cells. He feels, therefore, that the dissemination must take place by the lymphatic route. It is likely, however, that these tumors reach the skin both by the lymphatic and hematogenous route.

#### SUMMARY AND CONCLUSION

1. A report of a case of carcinoma of the stomach with numerous metastases to the skin, and with complete necropsy findings is presented.

2. The diagnosis of the nature of the skin lesion depends on biopsy for corroboration.

3. This case is also unusual because (1) it presents no lung involvement, and (2) the metastasizing cancer was of the scirrhus type.

4. The evidence points to both the hematogenous and the lymphatic route as means of transmission of the tumor cells.

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1004 MAY BUILDING

## THE HYDROGEN ION CONCENTRATION OF NASAL SECRETION IN CHILDREN WITH ACUTE CORYZA\*

### A PRELIMINARY REPORT

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THE object of the following experiment was to determine the range of normal variation of the hydrogen ion concentration in the nasal secretion of children and note any change which may occur in individuals during an attack of acute coryza

### MATERIALS AND METHODS

The nasal secretions for these determinations were obtained from children presented for annual or semiannual physical examination. They were from three months to thirteen years of age and no discrimination was made with respect to sex. Those children in whom no pathologic conditions were detected, were considered as normal and the  $P_H$  of their respective nasal secretions was recorded as such.

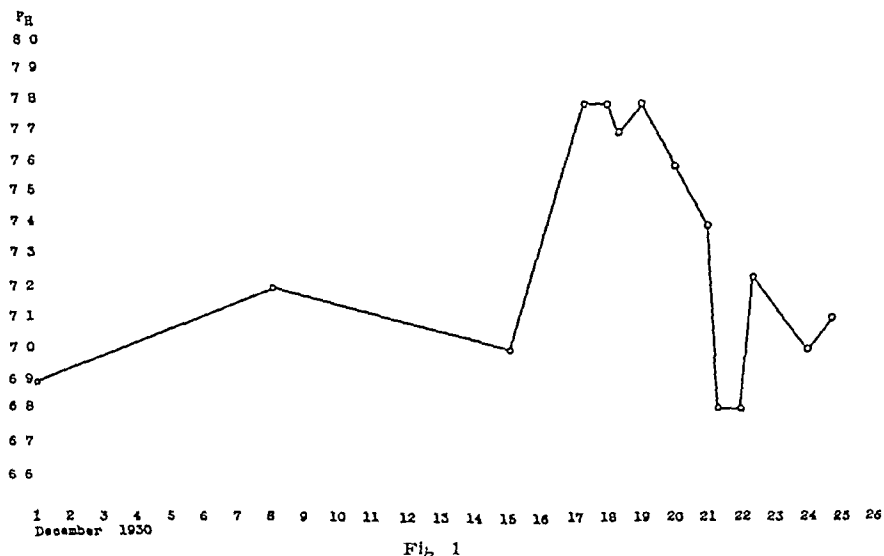
The  $P_H$  determinations of nasal secretion in acute coryza were made early in the disease. The children on whom these determinations were made represent selected cases in so far as no other pathologic condition could be detected. It should be emphasized that the  $P_H$  determinations were made on the nasal

secretion during the acute stage of the disease when the nasal discharge was thin, clear, and most profuse. In several instances  $P_H$  determinations were made on the same child, i.e., normal and during an attack of acute coryza.

With only slight modifications making it adaptable for nasal secretions, the methods used were the same as those developed by Clark<sup>1</sup>. The indicators used were phenol red having a  $P_H$  range of 6.7 to 8.2 and brom cresol purple from 5.6 to 6.9.

### RESULTS

The results obtained from these experiments are presented in Tables I and II, and in a graphic record (Fig. 1) of an individual case whose nasal secretion was examined over a period of several weeks, during which time an attack of acute coryza developed.



### COMMENT AND CONCLUSIONS

The  $P_H$  values recorded in Table I were obtained from children found to be of normal development in every respect. It will be noted that the  $P_H$  of their respective nasal secretions range from 6.8 to 7.2 with a mean value of  $7.03 \pm 0.021$ . While in some instances these results are slightly acid and in others slightly alkaline, they are practically within the neutral range.

Acute coryza is usually ushered in with several paroxysms of sneezing followed by a marked congestion of the nasal mucous membranes. During this period of congestion, there is quite a profuse thin, clear, watery discharge from the nose which may continue for forty-eight hours or longer. The  $P_H$  determinations were made on this watery discharge and the results obtained are recorded in Table II.

The  $P_H$  range of the nasal secretion in children with acute coryza extends from 7.2 to 7.9 with a mean value of  $7.69 \pm 0.039$ . From these results, it may be stated that in the early stages of acute coryza the nasal secretion gives an alkaline reaction.

TABLE I

## NORMAL

CASE	P <sub>H</sub>	CASE	P <sub>H</sub>
1	7.0	19	7.2
2	7.2	20	7.2
3	7.2	21	7.2
4	7.1	22	6.9
5	7.2	23	7.0
6	6.8	24	7.0
7	7.0	25	7.2
8	7.0	26	6.8
9	7.1	27	7.0
10	7.2	28	7.1
11	7.0	29	7.1
12	7.1	30	7.2
13	6.9	31	6.8
14	7.0	32	6.9
15	7.0	33	6.8
16	7.0	34	7.0
17	7.0	35	7.0
18	7.0		

$$\text{Mean} = 7.03 \pm 0.022$$

$$\text{St. D.} = 0.126$$

TABLE II

## ACUTE CORYZA

CASE	P <sub>H</sub>	CASE	P <sub>H</sub>
101	7.8	113	7.6
102	7.7	114	7.7
103	7.8	115	7.8
104	7.6	116	7.8
105	7.8	117	7.8
106	7.9	118	7.8
107	7.7	119	7.5
108	7.4	120	7.4
109	7.2	121	7.7
110	7.8	122	7.9
111	7.9	123	7.8
112	7.7	124	7.5

$$\text{Mean} = 7.69 \pm 0.039$$

$$\text{St. D.} = 0.189$$

The curve presented in Fig. 1 represents the P<sub>H</sub> or nasal secretion in a boy, aged eight years, who had been under observation for several weeks before the onset of an attack of acute coryza. In this case the normal P<sub>H</sub> of the nasal secretion on December 1 was 6.9, on December 8, 7.2, and on December 15, 7.0. During the night of December 16 this child had several attacks of sneezing and the next morning, December 17, a slight nasal discharge was noted which gave a P<sub>H</sub> value of 7.8. On the afternoon of the same day another determination was made and found to be the same, 7.8. The next morning, December 19, the P<sub>H</sub> of the nasal secretion was 7.6, and on December 20, 7.4. The following day, December 21, the mucous membranes of the nose were quite congested, were dry, and it was with some difficulty that sufficient secretion could be obtained for the P<sub>H</sub> determination. The results on this day were, AM 6.8 and PM 6.7. On December 22, the mucous membranes had a normal appearance and the nasal secretion gave a P<sub>H</sub> value of 7.2, on December 23, the P<sub>H</sub> was

7.0, and on December 24, 7.1. During the entire course of this attack the only therapeutic measures employed were complete rest in bed and plenty of fluids.

From these determinations it appears that the  $P_H$  of nasal secretion in normal children varies from 6.8 to 7.2 with a mean of  $7.03 \pm 0.021$ . These values are well within the neutral range of hydrogen ion concentrations. During the early stage of acute coryza, the  $P_H$  of nasal secretion extends from 7.2 to 7.9 with a mean of  $7.69 \pm 0.039$ . These results indicate an alkaline reaction.

#### SUMMARY

1. The hydrogen ion concentration of nasal secretion obtained from normal children extends from 6.8 to 7.2 with a mean of  $7.02 \pm 0.021$ . These results indicate practically a neutral reaction of the nasal secretion.

2. The hydrogen ion concentration of nasal secretion obtained from children during the early stages of acute coryza gave results extending from 7.2 to 7.9 with a mean of  $7.69 \pm 0.039$ . These  $P_H$  determinations indicate an alkaline reaction of the nasal secretion in this disease.

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106 EAST SIXTY-FIFTH STREET

### A CASE OF MARKED CREATINEMIA WITH RECOVERY\*

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IT IS now well established that the retention of considerable amounts of creatinine is associated with renal disease, and that the quantity of creatinine found in the blood is a fairly accurate index of the degree of renal impairment. For some time following the observations of Myers, creatinine values over 5 milligrams per 100 c.c. were thought to denote an early fatal termination. Exceptions were not long in appearing and one of these, reported by Myers,<sup>1</sup> was a patient with ureteral obstruction from stones whose blood creatinine was 21.2 mg. With relief of the obstruction the creatinemia returned to normal and the patient recovered.

The following report deals with an instance of marked creatinemia with recovery.

#### CASE REPORT

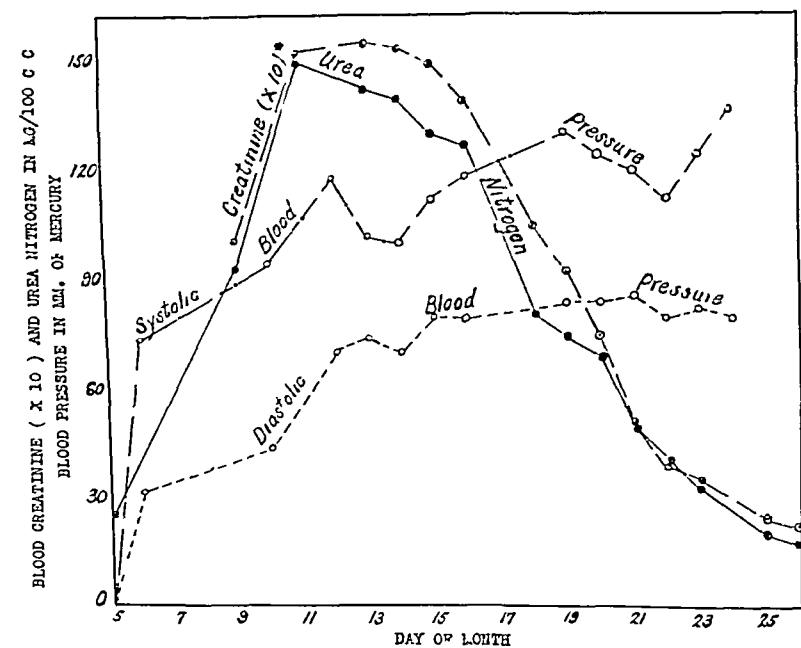
A colored woman, aged twenty-eight years, was admitted to the Cleveland City Hospital on April 4, 1932, at 6 A.M., complaining of asthma. Her past history was negative. Her present difficulty went back four years. During this time she has had attacks of asthma at irregular intervals. These attacks were associated with colds which are always present when the attacks occur. The attack which brought the patient into the hospital was of two days' duration.

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duration Patient said she has had some sort of "spells" during which time she would fall asleep for two days without being able to be aroused The first attack, five months previous to admission, came on after she had become extremely angry about something Two days before admission the patient became frightened by a fire

On physical examination the patient was found to be a well nourished and developed colored woman who presented the typical picture of an acute attack of asthma obstruction The patient was thoroughly conscious but "felt as if she were going to die" The other positive findings were as follows examination of lungs showed the typical findings of asthma The surprising findings were that the patient was pulseless, the systolic and naturally the diastolic pressure were so low that neither could be measured by a mercury manometer This finding was checked several times by several different men One half cubic centimeter of adrenalin was given, and within an hour the attack of asthma subsided and the patient said she felt better "all over" About four or five hours later, the patient had another feeling of impending death, she became restless and felt "very peculiar" She was then given the



\*Creatinine values multiplied by ten for graphing purposes

Chart 1

routine treatment for shock including hypertonic solutions of glucose intravenously, caffeine sodium benzoate, adrenalin, and normal saline solution intravenously She felt better During the entire period, the blood pressure could not be measured At 10 P M, or about sixteen hours after admission, her blood pressure was measured for the first time It was 74/32 The first dose of adrenalin had caused the asthma to disappear

The examination of the urine showed two plus albumin and the sediment was composed of hyaline and granular casts and a great many white blood cells Urea nitrogen was 27 mg per 100 cc Creatinine test was not made The fluid intake on the day of admission was 2300 cc, but the output was only 50 cc The red blood count, the white blood count, and hemoglobin estimation were of no significance

There was a very rapid rise of both urea nitrogen and creatinine to 142 mg and 15.4 mg per 100 cc respectively on April 13 (Chart 1) From this time, there was a slower fall of these nitrogen elements of the blood, i.e., while the height was reached in about eight days, the return to normal required twelve days

The blood pressure gradually rose but did not reach its highest point until April 20, or two weeks after admission

The output gradually increased until on April 15, it totalled 1660 cc and contained 12.8 gm of urea or about one half the normal amount. The creatinine in the blood was just receding from its high point. On April 26, the output was 3100 cc, the urea output was 21.7 gm, the urea nitrogen and creatinine were 18 mg and 23 mg respectively.

The phenolsulphonaphthalein output was zero on April 12. This was not repeated. The urine gradually cleared, and became negative.

The  $\text{CO}_2$  determinations varied from 43 to 64 volumes per cent.

*Comment*—The amazing fact about the course of this case is that in spite of the blood and other laboratory findings the patient was perfectly comfortable and conscious during the entire time of her hospital stay.

The exact cause of the findings is not known, however, it is suggested that due to the marked drop of blood pressure and the resultant disturbance in the blood flow through the kidneys, there was an accumulation of nitrogen metabolites in the blood together with the other evidences of kidney insufficiency and as the blood pressure rose to normal, all the evidences of kidney insufficiency disappeared. Whether the fall in blood pressure was due to an anaphylaxis as the result of the asthma obstruction is difficult to say.

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7016 EUCLID AVENUE

## THE NORMAL RANGE OF CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF HEALTHY NONPREGNANT WOMEN\*†

J. W. MULL, PH.D., AND A. H. BILL, M.D., CLEVELAND, OHIO

IN A PREVIOUS publication the authors<sup>1</sup> presented evidence, both from the literature and from their own work, that the generally accepted range of 9 to 11 mg Ca per 100 ml of serum is too low. The present study, in which the calcium and phosphorus was determined in 207 healthy nonpregnant women, fully confirms our earlier conclusions.

The same methods were employed as before. The calcium was determined by the method of Kramer and Tisdall<sup>2</sup> with slight modifications in technique as suggested by Collip and Clark,<sup>3</sup> Clark,<sup>4</sup> and our own experience. Tests were run from time to time throughout the course of the study by making multiple determinations on the same sample of serum, by recovering calcium added to the serum, and by determining the calcium in prepared solutions of known strength. These tests indicate an average error of from 1 to 2 per cent.

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Received for publication August 26 1932.

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Phosphorus was determined by the method of Kuttner and Cohen<sup>7</sup> as modified by Kuttner and Lichtenstein,<sup>8</sup> with some suggestions in handling from Dr. Shohl. Standards of different strength were used so that unknowns could always be read within 3 mm plus or minus of the standard used, set at 20 mm. Tests as described above indicate an average error of 5 to 6 per cent.

The subjects were nurses, hospital employees, and patients who came to our prenatal clinics, but were found not to be pregnant. Blood was drawn just before lunch in all cases except for the clinic patients, where it was taken just after lunch. It was drawn into centrifuge tubes, allowed to clot, and then

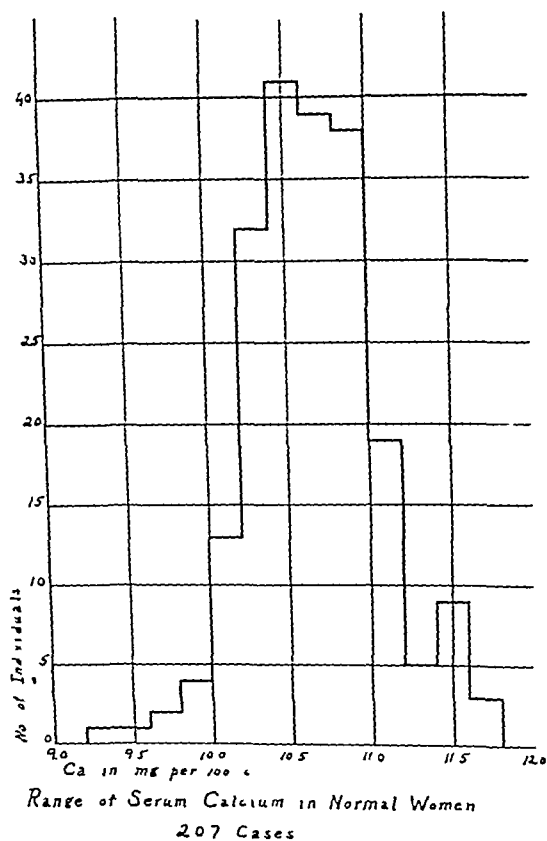


Fig 1

centrifuged. The serum was poured off, recentrifuged to remove all red cells, and used for the determinations.

In Table I the subjects have been grouped first on the basis of diet, those eating in the dormitory having a more or less standard diet, those at home eating a freely selected diet, and the outpatients being on practically a minimum diet. As regards calcium, the greatest difference in the averages of these three groups is 0.17 mg, while the probable error of this difference is 0.064 mg. In some cases this difference may be considered as significant, but we do not feel justified in so considering it here, where the analyses are made in biologic fluids.

The subjects were then regrouped on the basis of age into those below thirty years of age and those thirty or over. The selection of thirty was made

TABLE I  
CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF NORMAL WOMEN

GROUP	CALCIUM				PHOSPHORUS			
	NO IN GROUP	AVERAGE (mg/100 cc)	PROBABLE ERROR OF THE MEAN (mg/100 cc)	PROBABLE ERROR OF THE DIFFERENCE	NO IN GROUP	AVERAGE (mg/100 cc)	PROBABLE ERROR OF THE MEAN (mg/100 cc)	PROBABLE ERROR OF THE DIFFERENCE
Dormitory diet	134	10.63	0.025		132	3.74	0.022	
Independent diet	31	10.58	0.047		30	3.36	0.063	
Outpatients	42	10.75	0.040		41	3.40	0.041	
Greatest difference		0.17		0.064		0.38		0.063
Below 30 years—Nurses	142	10.70	0.021		109	3.84	0.021	
Outpatients {					32	3.48	0.049	0.054
Thirty years and over	65	10.54	0.038		62	3.31	0.041	
Difference		0.16		0.045		0.50		0.014
Hospital diet								
Below 30 years					97	3.82	0.022	
Thirty years and over					35	3.51	0.050	
Difference						0.31		0.054
Independent diet								
Below 30 years (nurses)					12	3.72	0.028	
Thirty years and over					27	3.13	0.058	
Difference						0.59		0.085
Below 30 years								
Hospital diet					97	3.82	0.022	
Independent diet					12	3.72	0.058	
Difference						0.10		0.063
Thirty years and over								
Hospital diet					35	3.51	0.050	
Independent diet					27	3.13	0.058	
Difference						0.38		0.078

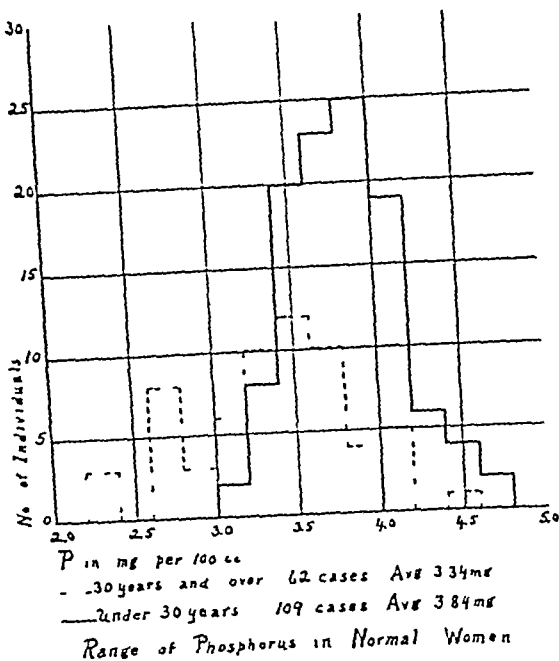


FIG 2

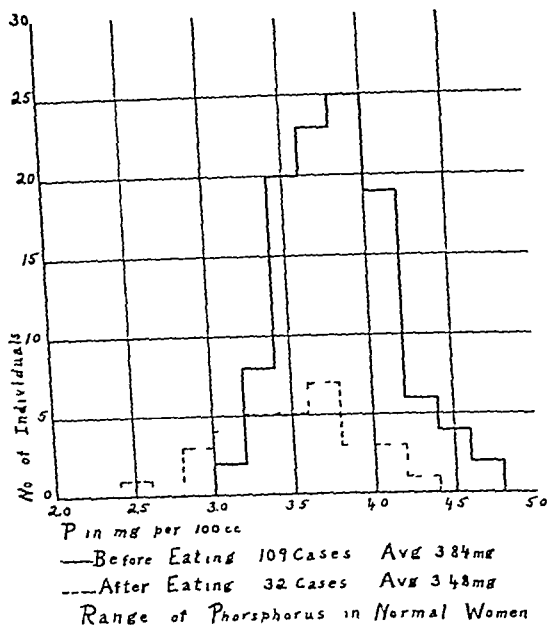


FIG 3

because it was the only significant point found when our subjects were grouped in ten year periods. The average difference in this case is 0.16 mg with a probable error of 0.045 mg. Again we do not feel justified in calling this a significant difference. From this we conclude that calcium is not materially affected by age or diet, so long as there is an adequate intake.

With phosphorus, however, a glance at Table I shows marked differences. On the first diet grouping the greatest difference of the averages given is 0.38 mg, while the probable error is only 0.063 mg. This difference is unquestionably significant. The age grouping brings out another factor, those under thirty are separated into nurses and outpatients, with widely differing averages. This is due to the fact that the former were taken before lunch and the latter after. It has been shown by Bollinger and Hartman,<sup>8</sup> Harrop and Benedict,<sup>9</sup> and others that there is a marked fall in the inorganic phosphorus of the blood dur-

TABLE II

CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF NORMAL NONPREGNANT WOMEN  
OUTPATIENT GROUP

ALL FIGURES GIVEN IN MG PER 100 ML SERUM

NAME	AGE	PARA	DATE	CA	P	NAME	AGE	PARA	DATE	CA	P
L W	30	1	5-27-31	10.22	3.75	N D L	22	1	12-15-31	11.14	4.04
			6-17-31	11.41	4.35				1-12-32	11.07	3.31
			8-12-31	10.68	2.72				Avg	11.10	3.67
			Avg	10.77	3.61						
A H	29	1	8- 3-31	10.56	3.10	R W	19	1	10-18-30	10.78	3.57
			9-14-31	11.23	3.60				12-13-30	10.52	2.93
			Avg	10.90	3.35				Avg	10.65	3.25
R G	19	1	4-24-31	10.91	2.53	N W	22	1	9-29-31	11.00	2.77
			5-15-31	10.84	3.10				10-20-31	11.11	3.51
			6- 5-31	10.60	2.83				11- 3-31	10.74	—
			Avg	10.78	2.82				Avg	10.95	3.14
I A	31	11	9- 9-31	11.26	—	L A	16	1	3- 5-32	10.42	4.02
			9-30-31	11.55	4.53				3-26-32	10.73	4.17
			Avg	11.40	3.53				Avg	10.58	4.10
A C	31	11	10-24-31	11.04	2.83	O P	19	11	11-13-31	10.71	3.29
			11-21-31	10.37	3.77				1-22-32	10.00	3.76
			Avg	10.70	3.30				Avg	10.35	3.52
L D	24	1	10-11-30	10.95	3.84	M G	27	1	5- 5-31	10.94	3.11
			12-20-30	10.17	3.41				5-26-31	10.86	2.91
			3-24-31	10.25	2.53				6-16-31	10.73	2.93
			Avg	10.46	3.26				8-18-31	10.68	2.72
									8-29-31	—	2.36
M H	23	1	10-28-30	10.16	—				Avg	10.80	2.81
			11-25-30	10.47	3.86						
			12-23-30	10.72	4.17						
			Avg	10.78	4.01						

Maximum deviation from the individual average Ca 0.64 mg P 0.88 mg

Average deviation from the individual average Ca 0.25 mg P 0.38 mg

mg carbohydrate metabolism. These groups, therefore, are not comparable. Those taken after eating cannot be considered here, and are not included in the subsequent groupings.

Between the compared age groups we find a difference in the averages of 0.50 mg, and a probable error of 0.044 mg. Here again is a significant difference. Either diet or age causes a difference in the serum phosphorus. In order to eliminate one of these two factors, those eating the standard hospital diet and those eating independently at home are both subdivided on the basis of age, and

in each case the difference found between the averages is significant. Then those below thirty are grouped by diet, and the difference is nominal. This seems to indicate that age is the important factor. The last group, that over thirty divided by diet, does not fall into line as expected. This failure may be due to a number of reasons, but the most probable seems to be that of individual variation. If age is the affecting cause, it is entirely probable that such a change would come not at any fixed year, as at thirty, but would vary with each subject.

TABLE III

CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF NORMAL NONPREGNANT WOMEN  
HOSPITAL GROUP

ALL FIGURES GIVEN IN MG PER 100 ML. SERUM

NAME	AGE	DATE	CA	P	NAME	AGE	DATE	CA	P
M L	38	6-18-31	10 34	3 16	M M	21	4-23-31	10 00	3 37
		10- 1-31	10 50	3 27			2- 3-32	10 18	3 51
		Avg	10 42	3 21			Avg	10 09	3 62
B	38	2-12-31	10 17	4 06	F B	26	8-20-31	10 58	4 31
		2-11-32	9 92	3 44			2-11-32	9 80	4 03
		Avg	10 04	3 75			Avg	10 19	4 17
B F	32	4-16-31	10 87	3 41	K	38	2- 5-31	9 33	3 19
		2-11-32	10 08	3 47			2-11-32	9 87	2 76
		Avg	10 47	3 44			Avg	9 60	2 97
D	43	2-12-31	9 90	3 66	J M	28	9-10-31	10 75	3 50
		2-11-31	—	3 82			2-17-32	9 60	4 40
		Avg	9 90	3 74			Avg	10 17	3 95
N C	25	5-21-31	10 57	4 10	W P	26	10-29-31	11 53	3 31
		2-17-32	9 55	3 69			2-18-32	9 70	3 49
		Avg	10 06	3 89			Avg	10 61	3 40
M L	28	8-14-31	10 52	4 00	M W	32	8-17-31	10 82	2 64
		2-18-32	9 90	4 53			1-18-32	9 87	2 44
		Avg	10 21	4 29			Avg	10 34	2 54
C A	26	6-11-31	11 06	3 86	N M	24	3- 3-32	9 94	4 18
		9-10-31	10 98	3 66			4- 7-32	9 80	3 76
		2-22-32	9 99	3 76			Avg	9 87	3 97
Avg			10 65	3 76					

Maximum deviation from the average Ca 0.92 mg P 0.45 mg

Average deviation from the average Ca 0.37 mg P 0.18 mg

studied. It would not be unlikely, therefore, to find disagreement in this group. We feel that the evidence favors the age factor, and that there is a decline in the phosphorus content of the serum of women, taking place, on the average, at around thirty years of age.

On the basis of the findings presented in Table I we have included all the 207 cases in the distribution curve given in Fig. 1. Over two-thirds, 81 per cent, fall within plus or minus 0.4 mg of the average, 10.6 mg per 100 ml. 94 per cent fall between 10.0 and 11.5 mg. On the basis of this evidence we claim that the normal range of serum calcium in healthy women, as found in Cleveland, is from 10.0 to 11.5 mg per 100 ml of serum.

In Fig 2 we have given the distribution curve for phosphorus for those thirty years and over, and for those under thirty. Over two-thirds of the latter, 80 per cent, fall within plus or minus 0.4 mg of the average, 3.8 mg per 100 ml. Ninety-two per cent fall between 3.2 mg and 4.4 mg, which may be considered the normal range for inorganic phosphorus for healthy women under thirty. The curve for those over thirty is, as would be expected, much less regular. The average found was 3.3 mg, and the range from 2.6 mg to 4.2 mg per 100 ml.

Fig 3 shows the difference due to carbohydrate metabolism. The 109 cases are the same as shown in Fig 2, under thirty, while the 32 cases are from the outpatient clinic, where the samples were drawn after lunch. There is a distinct difference in the averages and in the curves.

Tables II and III are given to confirm the validity of our findings. These tables show repeated tests on the same individual at intervals varying from two weeks to a year. They show a remarkable constancy in the calcium and phosphorus levels of a given individual. In Table II, outpatients, the maximum deviation of any finding from the average for that individual was only 0.64 mg for calcium and 0.88 mg for phosphorus, while the average deviation of any finding from the individual average was 0.25 mg for calcium and 0.38 mg for phosphorus.

In Table III, hospital group, there are some larger variations. It will be noticed that these occur between tests made during the early months of the year, our winter months, and those made during the summer or fall months. This led to the belief that there might be a seasonal variation in calcium. The evidence, however, proved contradictory and no such conclusion can be justified on the basis of our studies. No evidence was found of any seasonal variation in phosphorus.

#### SUMMARY

The calcium content of the serum of normal adult women is not affected by age or diet, provided there is an adequate intake.

The normal range of serum calcium in healthy women, under conditions in Cleveland, is from 10 to 11.5 mg per 100 ml of serum.

The inorganic phosphorus of the serum of healthy women is subject to an age variation, the normal range in women over thirty being lower than that for women under thirty.

Three and two-tenths to 4.4 mg per 100 ml of serum is the normal range of inorganic phosphorus for women under thirty, when the blood is drawn before eating.

The range for those over thirty is found to be from 2.6 to 4.2 mg per 100 ml of serum.

No conclusive evidence of seasonal variation was found in either the calcium or the inorganic phosphorus.

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## STUDIES ON GASTRIC MUCIN\*

### ITS EFFECT ON GASTRIC ACIDITY

DAVID H. ROSENBERG, M.D., AND LEON BLOCH, M.D. CHICAGO, ILL.

THE physiologic rôle of gastric mucus has in the past attracted relatively little attention. That it possesses a protective function was suggested by William Beaumont<sup>1</sup> in 1833 and reiterated by Claude Bernard,<sup>2</sup> Schiff,<sup>3</sup> Pavlov,<sup>4</sup> Klug,<sup>5</sup> and more recently by Whitlow<sup>6</sup>. Heidenhain<sup>7</sup> in 1879 ascribed to it the power of reducing gastric acidity. Pavlov<sup>4</sup> stated that "a perfectly pure juice may have its acidity reduced to the extent of 25 per cent by neutralization with mucus." Gies<sup>8</sup> in 1903 concluded that whereas precipitated mucoid shows practically no acid combining power, "in the hydration of mucoid by pepsin-acid . . . acid combines with the dissolved proteid products formed in the process." Extending the work of Gies, Foster<sup>9</sup> in 1907 observed that gastric mucus obtained by lightly scraping the mucosa of pigs' stomach, in the presence of pepsin is capable of combining with hydrochloric acid to a relatively marked degree. Further, Lim<sup>10</sup> noted that the concentration of mucus was higher than normal when the rates of gastric secretion (HCl output) were low. Because of its power to combine readily with free acid, its "protective, soothing lubricating rôle" and the fact that "its secretion or ingestion causes no chemical disturbance in the body and no unfavorable effect on gastro-intestinal secretory or motor activity," it was recently proposed by Fogelson<sup>11</sup> as an ideal antacid. Using Pavlov pouch dogs, Fogelson<sup>11</sup> found that gastric mucin, a substance prepared from the mucosa of hogs' stomach, has a high acid combining power. When administered to patients with chronic peptic ulcer, it resulted in complete relief of symptoms within three days of treatment. Kim and Ivy<sup>12</sup> later reported the prevention of ulcer formation in 17 dogs with biliary fistula by feeding 15 gm. of mucin twice daily.

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Pursuant upon Fogelson's interesting preliminary report, we undertook a comprehensive study of its clinical value and at this time, wish to report the effect of commercial mucin and some of its fractions upon the gastric acidity in eight patients with uncomplicated chronic peptic ulcer. Our results in the treatment of 28 patients are to be reported elsewhere.<sup>1-3</sup> The mucin preparations and the information pertaining thereto were furnished by Dr. David Klein, of The Wilson Laboratories.

#### GENERAL PROCEDURE

A Rehfuess tube was passed into the fasting stomach and retained throughout the period of observation. The stomach was emptied, the test meal given and at fifteen minute intervals 10 cc of gastric contents were aspirated and examined immediately for free and total acidity. At the termination of each aspiration, the residual content of the tube was forced back into the stomach and the end of the tube clamped. Individual series of observations were made on successive mornings.

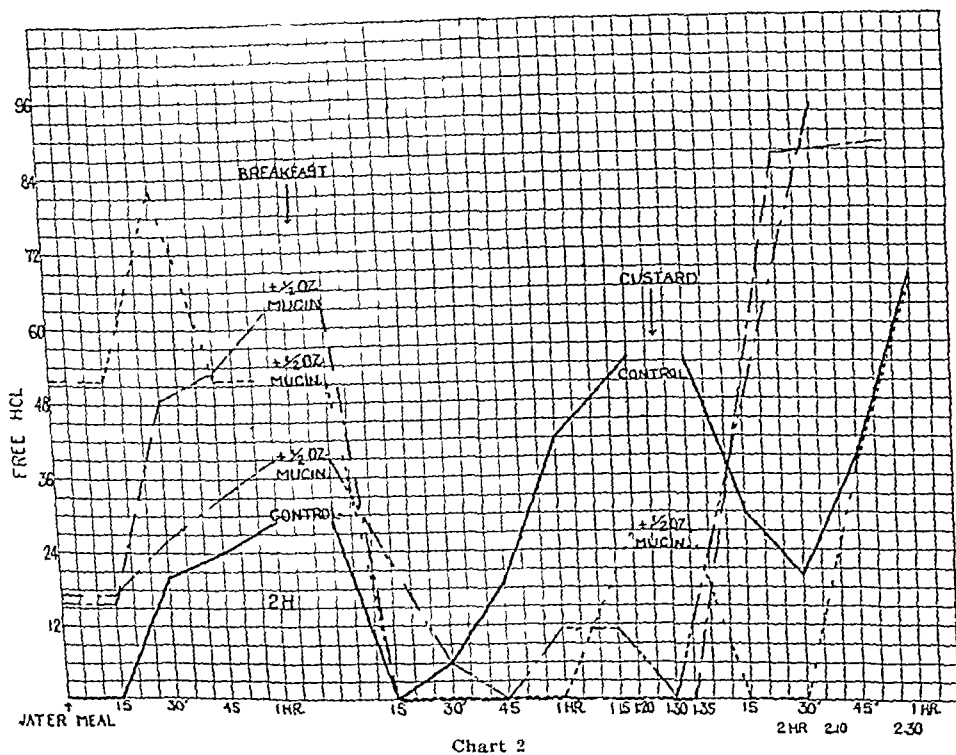
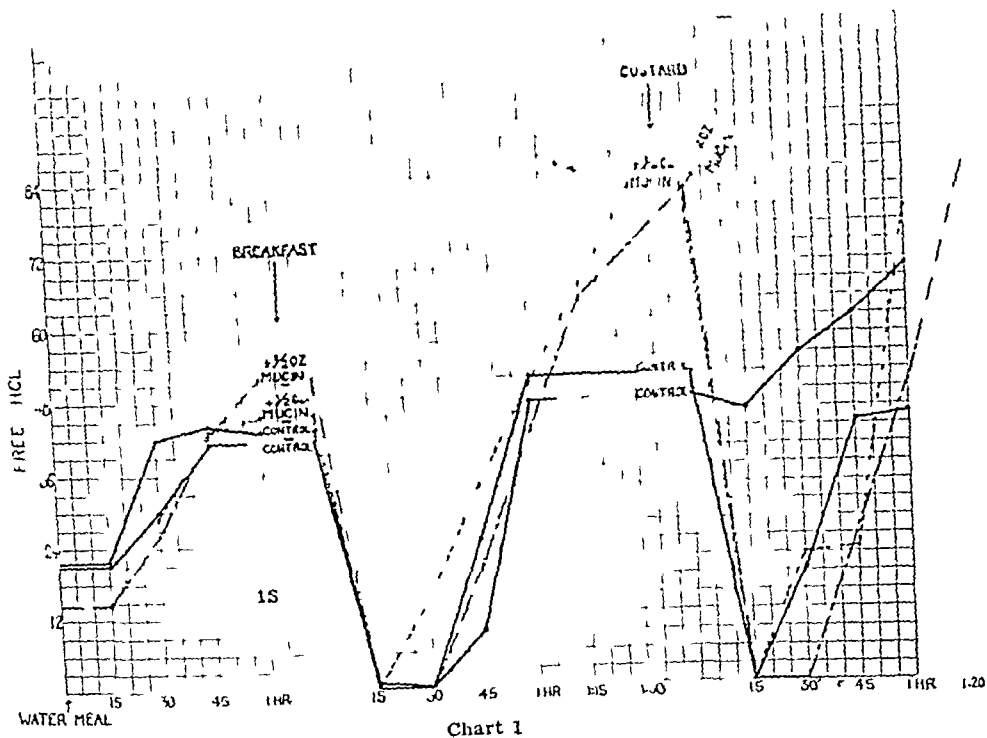
#### 1 ALTERATIONS IN THE ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN

With the tube in situ, gastric secretion was stimulated by having the patient drink 400 cc of water. One hour later a bland breakfast (qualitatively and quantitatively the same throughout the period of study) was served. Maintaining the usual ward ulcer routine, an interval feeding of three ounces of custard was given approximately two hours later. With one or more such series established as controls, one-half ounce of gastric mucin was added to the breakfast and to the custard. We hoped thereby to determine to what degree the buffering action of a bland feeding is enhanced by the addition of this amount the usual therapeutic dose, of mucin. Such studies were carried out upon three patients.

In patient 1S (Table I, Chart 1), the control breakfast on two occasions resulted in a complete absence of free HCl for one-half hour. By the addition of mucin, a similar result was obtained in one experiment, in another, free HCl was absent for one-fourth of an hour. The subsequent addition of custard had no effect in one control, but in the other there was an absence of free HCl for one-fourth of an hour. Mucin added to custard produced an absence of free acid for one-fourth and one-half hour respectively in two experiments. A mild or moderate secondary hyperstimulation was observed in three of four instances in which mucin was used.

In patient 2H (Table II, Chart 2), the results with mucin were much more striking. Whereas the control breakfast produced an absence of free HCl for one-fourth of an hour, with mucin no free acid was detected for one hour in one experiment and for one and one-half hours in two subsequent ones. In the latter two experiments a secondary hyperstimulation rapidly ensued. The later addition of custard to the control reduced the free HCl to a moderate degree, but custard together with mucin reduced the free acidity to zero for one-half hour, although at the three quarter hour period its value was the same as the control.





Charts 1 and 2—Gastric free acidity following one or more bland feedings with and without gastric mucin. Gastric secretion was stimulated by 400 c.c. water and breakfast given one hour later. Custard if used was given two hours after breakfast (Patients 1S and 2H).

TABLE I  
GASTRIC ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN  
(PATIENT 1S)

TEST MEAL	1/4 HR	1/2 HR	3/4 HR	1 HR	FEEDING	1/4 HR	1/2 HR	3/4 HR	1 HR	1 1/4 HR	1 1/2 HR	1 3/4 HR	2 HR	FEEDING	1/4 HR	1/2 HR	3/4 HR	1 HR	1 1/4 HR
400 cc Water	*TA 217	420	440	430	Breakfast	10	00	05	470	---	---	---	---	(ustard	450	540	600	680	---
(Control)	*TA 267	480	500	500	(Control)	340	445	450	775	---	---	---	---	(Control)	690	780	825	880	---
400 cc Water	TA 210	300	410	---	Breakfast	00	00	275	510	---	---	---	---	Custard	00	185	430	440	---
(Control)	TA 250	340	450	---	(Control)	300	470	710	825	---	---	---	---	(Control)	480	755	910	870	---
400 cc Water	TA 145	260	425	520	Breakfast	00	00	200	430	640	870	810	---	Custard	00	00	---	---	---
(Control)	TA 165	295	455	565	+ 1/2 oz mucin	610	620	520	740	910	820	970	---	+ 1/2 oz mucin	00	210	220	770	---
400 cc Water	TA 145	290	430	455	Breakfast	00	150	333	505	---	---	---	---	Custard	00	686	820	920	---
(Control)	TA 190	325	480	510	+ 1/2 oz mucin	470	407	628	700	---	---	---	---	+ 1/2 oz mucin	00	820	920	990	---

\*TA—Free acidity \*TA—Total acidity

TABLE II  
GASTRIC ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN  
(PATIENT 2H)

TEST MEAL	1/4 HR	1/2 HR	3/4 HR	1 HR	FEEDING	1/4 HR	1/2 HR	3/4 HR	1 HR	1 1/4 HR	1 1/2 HR	1 3/4 HR	2 HR	FEEDING	1/4 HR	1/2 HR	3/4 HR	1 HR
400 cc Water	TA 00	200	240	290	Breakfast	00	60	190	420	550	---	---	---	Custard	300	200	390	681
(Control)	TA 50	240	280	330	(Control)	240	390	550	700	916	---	---	---	(Control)	740	688	875	969
400 cc Water	TA 520	825	520	520	Breakfast	00	00	00	00	280	---	---	---	Custard	00	00	380	670
(Control)	TA 570	865	560	580	+ 1/2 oz mucin	517	500	500	766	940	---	---	---	+ 1/2 oz mucin	712	775	980	1120
400 cc Water	TA 570	865	560	580	+ 1/2 oz mucin	517	500	500	766	940	---	---	---	+ 1/2 oz mucin	712	775	980	1120
400 cc Water	TA 100	490	533	630	Breakfast	00	00	00	00	00	---	---	---	---	---	---	---	---
(Control)	TA 180	510	575	690	+ 1/2 oz mucin	500	600	630	625	---	---	---	---	---	---	---	---	---
400 cc Water	TA 170	260	330	390	Breakfast	---	60	00	114	113	---	---	---	---	---	---	---	---
(Control)	TA 200	290	360	430	+ 1/2 oz mucin	---	660	830	843	1000	1030	---	---	---	---	---	---	---

TABLE III  
GASTRIC ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN  
(PATIENT 3M)

TABLE III																			
GASTRO ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC ACID										GASTRO ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC ACID									
(PATIENT 3M)										(PATIENT 3M)									
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HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
FEEDING										FEEDING									
(Control)										(Control)									
Breakfast										Breakfast									
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In patient 3M (Table III), we endeavored to compare the effects of two feedings (breakfast and later custard) with a similar breakfast containing one-half ounce of mucin (no custard). The results were interesting, for while the mucin produced an achlorhydria for three-fourths of an hour compared with one-fourth of an hour for the control, the final readings two and one-half hours after each breakfast were the same. No secondary hyperstimulation occurred.

## 2 COMPARATIVE INFLUENCE OF COMMERCIAL MUCIN AND "PURE" MUCIN

One brand of commercial mucin, according to the manufacturer, contains approximately 60 per cent "pure" mucin and 40 per cent peptone and ash.

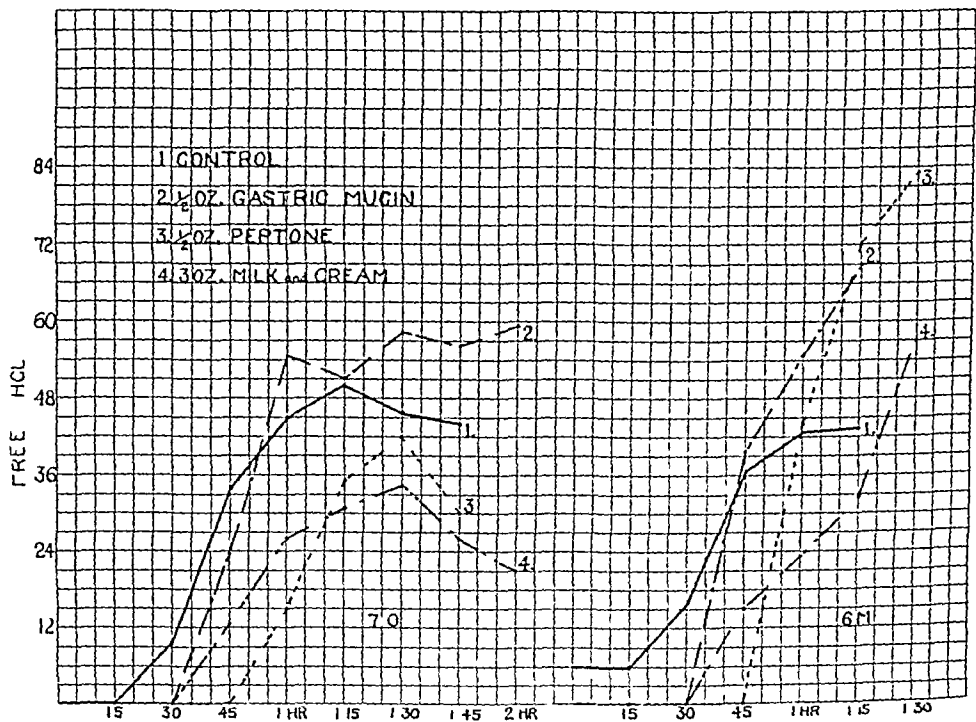


Chart 3—Comparative effect of gastric mucin, peptone-phosphate fraction and milk and cream mixture. 500 cc oatmeal gruel was used as control and as gastric stimulant (Patients 70 and 6M).

material. It was desirable therefore, to compare the relative antacid properties of commercial mucin ("gastric" mucin) with that of the "pure" mucin. Five hundred cubic centimeters of warm oatmeal gruel were used as the standard test meal (Crohn and Reis<sup>13</sup>). Accepting the response to such a meal as our control, the respective mucin preparation was added, in all instances, aspirations were continued until the stomach was empty. Observations were made upon two patients (Tables IV and V, Chart 3).

In both individuals one-half ounce of gastric mucin effected an absence of free HCl which persisted for one-half hour. "Pure" mucin produced the same result in patient 4B, but in patient 5M, the achlorhydria lasted for only one-fourth of an hour. To observe the effect of a larger dose, one ounce

TABLE IV  
COMPARATIVE EFFECT OF GASTRIC MUCIN, PURE MUCIN, PEPTONE AND MILK AND CREAM  
UPON GASTRIC ACIDITY  
(PATIENT 1B)

TEST MEAL		$\frac{1}{4}$ HR.	$\frac{1}{2}$ HR.	$\frac{3}{4}$ HR.	1 HR.	$1\frac{1}{4}$ HR.	$1\frac{1}{2}$ HR.	$1\frac{3}{4}$ HR.	2 HR.	$2\frac{1}{4}$ HR.
Oatmeal gruel (Control)	FA TA	25 120	133 214	210 370	150 570	560 690	560 690	---	640 770	610 760
Oatmeal gruel + $\frac{1}{2}$ oz gastric mucin	FA TA	00 205	00 320	155 625	162 822	590 922	725 1025	920 1075	Empty	
Oatmeal gruel + $\frac{1}{2}$ oz pure mucin	FA TA	00 195	00 350	130 530	530 825	735 995	780 1000	820 1030	805 990	1012 1187
Oatmeal gruel - 1 oz. gastric mucin	FA TA	00 410	00 450	00 600	00 790	315 1015	520 1190	845 1180	900 1160	
Oatmeal gruel - 1 oz pure mucin	FA TA	00 345	00 510	20 670	340 950	450 930	690 1050	810 1044	787 987	
Oatmeal gruel + $\frac{1}{2}$ oz peptone	FA TA	00 300	00 350	00 520	00 740	295 905	555 944	695 1080	870 1100	
Oatmeal gruel - 3 oz milk and cream	FA TA	00 160	00 210	00 435	160 560	300 770	450 730	700 925	665 980	
Oatmeal gruel (Control)	FA TA	00 130	100 340	290 530	366 611	380 620	517 733	345 550	440 650	

TABLE V  
EFFECT OF GASTRIC MUCIN AND PURE MUCIN UPON GASTRIC ACIDITY  
(PATIENT 5M)

TEST MEAL		$\frac{1}{4}$ HR.	$\frac{1}{2}$ HR.	$\frac{3}{4}$ HR.	1 HR.	$1\frac{1}{4}$ HR.	$1\frac{1}{2}$ HR.	$1\frac{3}{4}$ HR.
Oatmeal gruel (Control)	FA TA	55 220	410 530	580 700	435 560	410 590	Empty	
Oatmeal gruel + $\frac{1}{2}$ oz gastric mucin	FA TA	00 210	00 350	120 620	489 789	490 740	437 688	606 794
Oatmeal gruel + $\frac{1}{2}$ oz pure mucin	FA TA	00 275	310 550	310 340	556 744	550 730	220 500	512 706

TABLE VI  
COMPARATIVE EFFECTS OF GASTRIC MUCIN, PEPTONE AND MILK AND CREAM UPON GASTRIC  
ACIDITY  
(PATIENT 6M)

TEST MEAL		$\frac{1}{4}$ HR.	$\frac{1}{2}$ HR.	$\frac{3}{4}$ HR.	1 HR.	$1\frac{1}{4}$ HR.	$1\frac{1}{2}$ HR.
Oatmeal gruel (Control)	FA TA	60 270	160 400	370 620	430 720	436 640	Empty
Oatmeal gruel + $\frac{1}{2}$ oz gastric mucin	FA TA	00 460	00 380	400 890	550 1020	690 910	Empty
Oatmeal gruel + $\frac{1}{2}$ oz peptone	FA TA	00 510	00 580	00 735	440 840	720 940	830 970
Oatmeal gruel + 3 oz. milk and cream	FA TA	00 310	00 290	150 420	230 500	325 585	557 700

each of gastric mucin and of "pure" mucin was given on successive days to patient 4B (Table IV). An achlorhydria lasting practically three-fourths of an hour was obtained with the "pure" mucin, contrasted with one hour when gastric mucin was administered. In all of these experiments, the achlorhydric period was superseded by a rapid rise in free HCl, the readings becoming equal to the control at the end of one hour when one-half ounce of either form of mucin was added and at the end of one and one-half hours when one ounce was given. A secondary hyperstimulation occurred in all mucin studies on patient 4B.

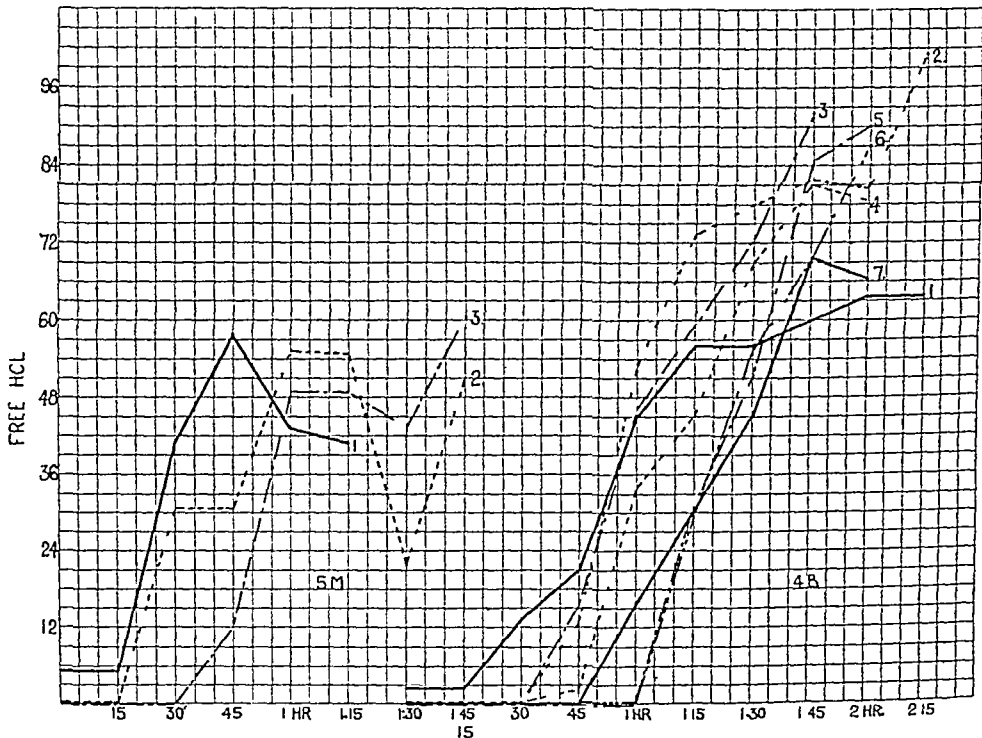


Chart 4—Comparative effect of (1) Control (500 c.c. oatmeal gruel) (2) One-half ounce pure mucin (3) One-half ounce gastric mucin (4) One ounce pure mucin (5) One ounce gastric mucin (6) One-half ounce peptone-phosphate fraction (7) Three ounces milk and cream mixture. The substances tested were added to 500 c.c. oatmeal gruel (Patients 5M and 4B).

### 3 NEUTRALIZING CAPACITY OF THE PEPTONE FRACTION

This phase of our study was conducted upon three patients in a manner similar to Part 2. One-half ounce of the peptone fraction was compared with one-half ounce of gastric mucin (Tables IV, VI, VII, Charts 3 and 4).

When the peptone fraction was added to the test meal, an absence of free HCl was noted for three-fourths of an hour in two patients (6M and 7O) and for one hour in the third (4B) as compared with one-half hour when gastric mucin was tested. In the third patient (4B) the response to one-half ounce of peptone corresponded to that of one ounce of gastric mucin. The subsequent rapid appearance of free HCl previously remarked upon, was likewise observed in this group. Mild hyperstimulation was present in two patients (4B, 6M).

#### 4 THE ACID COMBINING POWER OF A MILK AND CREAM MIXTURE A COMPARISON WITH GASTRIC MUCIN AND ITS DERIVATIVES

The uniformity with which frequent milk and cream feedings bring about a rapid remission in many instances of uncomplicated peptic ulcer, as well as its recognized capacity for combining with free HCl, suggested a comparative study with gastric mucin and its fractions. Three ounces of milk and cream (equal parts) and one half ounce each of gastric mucin, pure mucin, and peptone were tested in four patients. The method employed was the same as that in Parts 2 and 3 (Tables IV, VI, VII, VIII, Charts 3 and 4).

A milk and cream mixture of an amount equal to that used in routine ulcer management produced a total absence of free acid for one half hour in three patients and for three fourths of an hour in one. In three instances (6M, 7O, 8B) the readings at corresponding intervals were persistently less than then controls throughout the entire period of observation, in the fourth case they were less than the control for one hour. The rise in free acidity was not as rapid as that following mucin or its derivatives and furthermore, no secondary hyperstimulation appeared.

Contrasting these results with the ones obtained following the mucin products reveals interesting data, to wit: (1) Gastric mucin established an achlorhydria for one half hour in the three patients studied (4B, 6M, 7O). The same obtained after milk and cream in three cases, but in the fourth was prolonged to three-fourths of an hour. Further, the acid combining power of milk and cream was more sustained than that of gastric mucin. (2) The achlorhydria following "pure" mucin (4B, 8B) may be considered

TABLE VII  
COMPARATIVE EFFECTS OF GASTRIC MUCIN, PEPTONE AND MILK AND CREAM UPON GASTRIC ACIDITY  
(PATIENT 7O)

TEST MEAL		$\frac{1}{4}$ HR.	$\frac{1}{2}$ HR.	$\frac{3}{4}$ HR.	1 HR.	1 $\frac{1}{4}$ HR.	1 $\frac{1}{2}$ HR.	1 $\frac{3}{4}$ HR.	2 HR.
Oatmeal gruel (Control)	FA	0 0	10 0	34 0	45 0	50 0	46 0	44 0	---
	TA	19 0	29 0	66 0	74 0	67 5	68 0	65 0	---
Oatmeal gruel + $\frac{1}{2}$ oz. gastric mucin	FA	0 0	0 0	23 5	55 0	51 0	58 5	56 0	59 0
	TA	35 5	51 0	83 5	104 0	92 0	92 0	98 0	97 0
Oatmeal gruel + $\frac{1}{2}$ oz. peptone	FA	0 0	0 0	0 0	15 0	35 0	42 0	30 0	34 0
	TA	50 0	58 0	64 5	88 0	95 0	90 0	80 0	76 0
Oatmeal gruel + 3 oz. milk and cream	FA	0 0	0 0	13 0	26 0	31 0	34 5	26 0	21 0
	TA	21 0	45 0	46 5	55 0	64 0	73 5	57 0	54 0

TABLE VIII  
COMPARATIVE EFFECT OF PURE MUCIN AND MILK AND CREAM UPON GASTRIC ACIDITY  
(PATIENT 8B)

TEST MEAL		$\frac{1}{4}$ HR.	$\frac{1}{2}$ HR.	$\frac{3}{4}$ HR.	1 HR.	1 $\frac{1}{4}$ HR.	1 $\frac{1}{2}$ HR.	1 $\frac{3}{4}$ HR.	2 HR.	2 $\frac{1}{4}$ HR.	2 $\frac{1}{2}$ HR.
Oatmeal gruel (Control)	FA	3 0	30 0	36 0	51 0	58 3	55 0	---	63 0	---	---
	TA	27 0	53 0	63 0	70 0	75 0	72 0	---	93 0	---	---
Oatmeal gruel + $\frac{1}{2}$ oz. pure mucin	FA	0 0	0 0	25 0	24 0	36 0	51 0	56 0	71 5	85 5	74 0
	TA	43 0	60 0	76 0	82 5	92 0	102 0	107 0	105 5	104 4	99 0
Oatmeal gruel + 3 oz. milk and cream	FA	0 0	0 0	10 0	31 0	33 0	39 5	---	---	---	---
	TA	19 0	32 0	48 0	75 0	70 5	77 5	---	---	---	---

as of approximately the same duration as milk and cream, but again, the neutralizing effect was more prolonged with the latter (3) Peptone effected a more extended absence of free HCl than did milk and cream (three-fourths to one hour compared with one-half to three-fourths hour) in the three cases studied (4B, 6M, 7O), although in two of these, the neutralizing action was less sustained than when using milk and cream. In three of four patients (4B, 6M, 8B) mucin or its derivatives was productive of a hyperstimulation, an end-result not found with milk and cream.

#### DISCUSSION

In our studies on patients with chronic peptic ulcer, the achlorhydria following one-half ounce of gastric mucin, persisted for one-fourth to one and one-half hours in thirteen experiments, not exceeding one-half hour in the majority, although the total average was only forty minutes (Table IX). Giving one ounce of gastric mucin prolonged the period of achlorhydria to one hour in patient 4B, doubling the effect obtained with the smaller dose. With one-half ounce of gastric mucin, the free acidity was less than that of the controls for a total period of one-half to one and one-half hours, in the majority being one-half to three-fourths of an hour, including the period of absence of free HCl. That the neutralizing substances constituting 40 per cent of mucin play an important rôle in its antacid properties has been adequately demonstrated, since one-half ounce of the peptone ash fraction effected an achlorhydria for one-half to one hour in three instances. In Fogelson's experiments on Pavlov pouch dogs, achlorhydric periods as long as five to seven hours were observed when one-half ounce of gastric mucin together with one pound of meat was given. This disparity between our results and those of Fogelson may be dependent upon the prolonged buffering action of the meat.

Our studies on patients further corroborate the secretagogue action noted by Fogelson<sup>11</sup> on dogs and confirmed by Ivy and Kim,<sup>14</sup> for, of 21 experiments with mucin and its derivatives, a secondary hyperstimulation of a mild or moderate degree was present in 14, an incidence of 66 per cent (Table IX). Since the completion of our work, Rivers, Vanzant and Essex<sup>15 16</sup> reported the finding, in some of their patients, of a marked elevation in the acidity in association with an increased gastric secretion, the same being observed in dogs subsequent to the feeding of a regular meal containing 10 gm of mucin. The resulting curves simulated those following histamine stimulation. Three of four lots of mucin gave all the biologic tests for histamine, but none was detected in the fourth\*. More recently, Brown, Cromer, Jenkinson and Gilbert<sup>17</sup> reported "no marked secretagogue effect" from their mucin preparation.

The comparative results following the milk and cream mixture are particularly enlightening, despite the paucity of patients studied. Free acid was absent for one-half to three-fourths of an hour after the administration of the milk and cream, comparing favorably with the average response to one-half ounce of gastric mucin. The free acidity was less than that of the controls (at corresponding intervals) for one to one and three-fourths hours including

\*The mucin used by Rivers, Vanzant and Essex was of a different source from that used by us. The presence of histamine in mucin remains an open question.



TABLE IX  
STUDY OF LIGHT PATHWAYS

[illegible]

the period of achlorhydria, thereby showing a more prolonged neutralizing effect than one-half ounce of gastric mucin. Moreover, in no case did the milk mixture produce a hyperstimulation (Table IX). Such findings lead us to conclude that the greater virtue of gastric mucin when and if ultimately proved, must rest upon some factor other than its antacid property, such as a demulcent action, functioning as a protective coating to the ulcer, or upon some hitherto unknown or unsuspected factor.

#### CONCLUSIONS

1 The acid combining power of gastric mucin varies in the same or different individuals. The average period of achlorhydria produced by one half ounce of commercial mucin lasts forty minutes, the maximum observed is one and one-half hours.

2 The peptone ash fraction plays a considerable rôle in the antacid property of commercial mucin (gastric mucin).

3 The acid combining power of one-half ounce of gastric mucin is greater than that of a bland feeding.

4 In the majority of experiments in which gastric mucin or its derivatives were tested, a mild or moderate secretagogue action is demonstrable.

5 One-half ounce of gastric mucin produces an average achlorhydric period which compares favorably with that of a three ounce mixture of milk and cream but has the disadvantage of a secretagogue action.

6 The possible virtue of gastric mucin in peptic ulcer therapy is dependent upon some factor other than its antacid property such as a demulcent action.

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# LABORATORY METHODS

## A MODIFIED METHOD FOR DETERMINING ORGANIC PHOSPHORUS\*

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THERE has been much controversy concerning the probable presence of a protein other than bioplasm stored for the purpose of catabolism which has been designated as "reserve" or "deposit." Cahn and Bonat, in 1927, stated that the phosphorus contents of the isolated proteins of the liver of emaciated dogs, guinea pigs, and rabbits were greater than those of the corresponding proteins of respective obese animals. These results were interpreted as indicating the presence of reserve protein. In attempting to duplicate this work, I developed a modified method for determining organic phosphorus in small quantities of protein which I recommend as being quite satisfactory, and with which this paper is concerned.

The determination of organic phosphorus consists of (1) ashing the organic material, (2) converting the phosphorus to a definite chemical state, (3) getting the phosphorus into solution, and (4) applying a quantitative method.

The principal methods for ashing organic material are the wet and the dry. Neumann, Hartwell, Bosworth, and Kellogg, and Meigs, Blatherwick, and Cary used equal parts of concentrated nitric and sulphuric acids for ashing protein. Bauman stated that Raper, Taylor and Miller, Hartwell, Bosworth, and Kellogg preferred the dry ashing method. Bauman demonstrated errors in organic phosphorus determinations resulting from losses by volatilization and failure to convert all phosphorus to orthophosphoric acid. Hillebrand and Lundell demonstrated that all phosphorus could be safely converted to orthophosphoric acid only if heated in excess of concentrated sulphuric acid and that no volatilization would occur at a temperature of 150° C or below. Schulz ashed feces by means of digestion with concentrated sulphuric acid, and then added concentrated nitric acid drop by drop.

For the determination of phosphorus in solution, Neumann, in 1902, introduced the titrimetric method which Taylor and Miller, in 1914, improved

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Gibson and Estes, in 1909, introduced the uranium acetate method, Rapei, in 1914, described a gravimetric method using lead phosphomolybdate as the end product, King, in 1918, used  $Mg_2P_2O_7$  as the end point, Jones and Perkins, in 1923, used  $MgNH_4PO_4$  as the end point and dried the product to constant weight by the use of a vacuum at room temperature. Blooi, in 1915, introduced the nephelometric method, Greenwald, in 1915, introduced the first colorimetric method, the end point of which was really a yellow suspension rather than a true color. Bell and Dorsey, in 1920, introduced a method which in principle depended on the reduction of phosphomolybdic acid by hydroquinone in an alkaline medium. Briggs, in 1922, and again in 1924, offered modifications. Fiske and Subbarow, in 1925, demonstrated the errors of all existing colorimetric methods and offered a procedure which consisted of the reaction of orthophosphoric acid, ammonium molybdate, sodium sulphite, and 1-2-4 aminonaphthol sulphonic acid in 0.5 N sulphuric acid medium. Only five minutes were required for the development of the color, which was quite stable. Berggren has recently applied this method for determining phosphorus in casein.

#### PROCEDURE

After many attempts to determine phosphorus quantitatively in small samples of isolated protein, without obtaining satisfactory checks, the following procedure was finally adopted.

The ammonium molybdate used in the colorimetric method of Fiske and Subbarow was dissolved in water and the acid required to make the final volume 0.5 N was calculated and utilized in the ashing process. A quantity of powdered protein containing from 0.2 to 0.6 mg. of phosphorus was carefully weighed and washed with double distilled water into a hard glass test tube. One and four-tenths cubic centimeters of concentrated sulphuric acid was added and the tube was placed overnight in an electric oven kept at  $100^\circ C$ , during which time digestion occurred and all the water evaporated. The next morning the tube was placed in a support, a few drops of water were added and a method was arranged to keep the mouth of the tube cooled. This was done by making a U in the center of a glass tube, blowing a bulb at the bend of the U which would occlude about two-thirds of the mouth of the test tube, and attaching a spicule of glass at the most dependent portion of the bulb which extended slantingly to the side of the test tube (Fig. 1). Through this apparatus cool water flowed continually. The solution in the test tube was heated by means of a microburner until steam appeared and the flame was adjusted to keep the temperature constant. Then concentrated nitric acid was added drop by drop, the reaction resulting from one drop was allowed to subside before the next drop was added. When the solution had become yellow, a small quantity of aluminum sulphate was added which acted as a catalyst, and the procedure was continued until the solution was colorless. Then the contents were washed into a 100 cc. volumetric flask and the colorimetric method of Fiske and Subbarow was carried out, using the 2.5 per cent ammonium molybdate solution made up with water rather than with the 5 N sulphuric acid except in the case of the standard.

## COMMENT

Digestion of the protein by sulphuric acid may be effected by water-bath. The few drops of water added to the digested solution served as a thermometer during oxidation. The appearance of white fumes would indicate that the temperature was approximately  $150^{\circ}\text{C}$  and the flame should be lowered. A catalyst was necessary to destroy at a low temperature the color formed

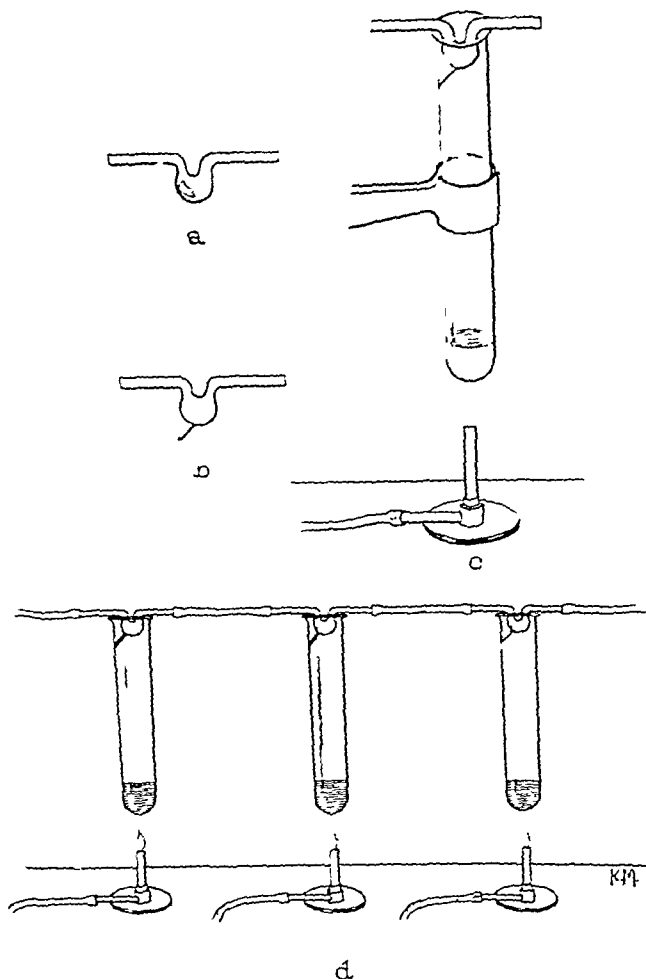


Fig 1—*a* U tube converted into a cooling apparatus *b*, cooling bulb equipped with a glass spicule *c* a single apparatus for digesting organic material and *d* three specimens being digested simultaneously

by a reaction between the benzene ring of the protein and the nitric acid. Mercuric sulphate was the best catalyst but it interfered with the colorimetric method used. A mark should be placed on the test tube to indicate  $1 \pm e e$  so that the concentrated sulphuric acid used during the oxidation can be replaced before applying the colorimetric test. The spicule of glass on the bulb was necessary to lead the condensed fluid gently back, thus avoiding an explosive

reaction which would result in the loss of some of the solution. Dozens of specimens can be digested overnight and about three specimens can be oxidized simultaneously.

The accuracy of the method of ashing was based on obtaining checks in duplicate determinations on the same powdered protein, and on recovering a known amount of phosphorus that had been added to the protein. The average result of four phosphorus determinations was used as the amount of phosphorus in the protein to which a known quantity was added.

## RESULTS

LIVER PROTEIN	PHOSPHORUS, PER CENT	AVERAGE, PER CENT
Sample 1	0.212	0.212
	0.212	
	0.209	
	0.213	
Sample 2	0.360	0.365
	0.369	
	0.370	
	0.363	

Five and two cubic centimeters of standard phosphate solution were added to known weights of liver protein, sample 1, containing 0.212 per cent phosphorus.

PHOSPHORUS ADDED, MG	PHOSPHORUS RECOVERED, MG	AVERAGE MG
0.4	0.404	0.399
	0.397	
	0.395	
	0.400	
0.16	0.161	0.158
	0.157	
	0.159	
	0.157	

## CONCLUSION

This method for determining phosphorus fulfills the requisites of Bauman to prevent losses by volatilization, and the conditions of Hillebrand and Lundell for complete conversion of all phosphorus to orthophosphoric acid. It is rapid, especially adapted to the use of small samples of organic material, and has an accuracy of  $\pm 2$  per cent.

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# SEPARATION AND DETERMINATION OF BISMUTH AND ARSENIC IN BIOLOGIC MATERIAL\*

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DURING an investigation of the excretion of arsenic by patients who had received bismuth previously, or who were receiving both arsenic and bismuth simultaneously or intermittently, the problem arose whether the amount of each of these elements could be determined in the same specimen of blood

## METHOD

Hanzlik, Mehlens<sup>1</sup> and coworkers studied the excretion of bismuth in patients who received bismuth alone, analytical complications from arsenic were thus absent

In the experiments here reported hydrazine sulphate was adopted as the most suitable reagent for the reduction of pentavalent arsenic. Since Jannasch and Seidel<sup>2</sup> introduced hydrazine sulphate for the separation of arsenic from other metals in the analysis of minerals, many other investigators<sup>3, 4, 5, 6</sup> have used hydrazine sulphate for their quantitative microanalysis of arsenic

The use of hydrazine sulphate not only permits the determination of bismuth in the residue from the arsenic distillation but at the same time replaces with great advantage the less stable  $\text{FeCl}_2$  (see Maechling and Flinn<sup>7</sup>)

With adequate modifications the distillate can be used for the colorimetric or volumetric<sup>8</sup> determination of arsenic

## PROCEDURE

The following procedure was adopted for the separation and determination of arsenic and bismuth in blood. 5 cc of blood mixed with known amounts of arsenic and bismuth were oxidized by means of 30 cc of a mixture of nitric and sulphuric acids (9:1), first in casserole covered with watch glasses on the water-bath and then in Kjeldahl flasks with the addition of 30 cc of concentrated sulphuric acid. The colorless acid digest was diluted with 70 cc of water and boiled to destroy traces of nitrosyl sulphuric acid, the Kjeldahl flask with the digest was connected to an Allihn form of condenser, a safety bulb being placed between the condenser and the receiving cylinder

A mixture of 10 gm of  $\text{NaCl}$ , 1 gm of  $\text{NaBr}$  and 1 gm of hydrazine sulphate was added at once to the acid digest in the Kjeldahl flask and the

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TABLE I  
ARSENIC AND BISMUTH RECOVERIES FROM BLOOD

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND
GAMMA	GAMMA	GAMMA	MG	MG
None	11	None	None	None
None	10	None	None	None
None	15	None	None	None
100	140	97	None	None
100	142	99	None	None
100	144	101	None	None
None	16	None	10	10
None	43	None	10	108
None	46	None	10	116
100	143	100	10	10
100	139	97	10	097
100	148	106	10	11
100	144	101	10	10
100	139	97	10	097

\*In the determinations marked with an asterisk in the tables 15 gm of NaCl and 15 gm NaBr and 15 gm hydrazine sulphate were used in all other instances 10 gm of NaCl 10 gm of NaBr and 10 gm of hydrazine sulphate were used for the reduction and distillation of arsenic

TABLE II  
ARSENIC AND BISMUTH RECOVERIES FROM WATER

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND
GAMMA	GAMMA	GAMMA	MG	MG
None	40	None	10	11
None	39	None	10	09
None	42	None	10	09
None	39	None	10	10
None	57*	None	20	19
None	55*	None	20	23
20	251*	196	20	21
20	239	199	04	04
25	291	251	05	05
25	291	251	10	09

arsenic distilled off in the usual way, starting with a very low flame and passing air at the slowest possible rate

The arsenic trichloride distilled over and was caught and oxidized in the receiving cylinder containing 10 c.c. of concentrated nitric acid and 5 c.c. of bromine water. The resulting solution was evaporated to dryness on the water-bath and the pentavalent arsenic determined colorimetrically.

To the residue in the Kjeldahl flask containing the bismuth, 20 c.c. of concentrated nitric acid were added in order to decompose the excess of hydrazine sulphate. The mixture was heated until the appearance of white fumes. The digest was cooled, diluted with water and boiled once more until white fumes reappeared. Bismuth was determined in this solution by Leonard's method. Citric acid was added to the cold solution in every case, because of the presence of iron in blood. The bismuth standard was acidified by means of sulphuric acid in order to obtain similar conditions.

TABLE III  
RECOVERIES OF ARSENIC AND BISMUTH IN WATERY SOLUTION

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND
GAMMA	GAMMA	GAMMA	MG	MG
None	5.7*	None	2.0	1.9
None	5.5*	None	2.0	2.3
20	25.1*	19.6	2.0	2.1

TABLE IV  
ARSENIC AND BISMUTH RECOVERIES FROM BLOOD IN THE PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG	MG	MG
None	3.8	None	None	None	0.2
None	4.0	None	None	None	0.2
None	4.1	None	0.2	0.2	1.0
None	4.4	None	0.4	0.4	1.0
None	3.8	None	0.4	0.4	0.5
10.0	13.5	9.4	None	None	0.5
10.0	13.7	9.6	None	None	0.5
10.0	13.8	9.7	0.2	0.2	0.1
10.0	13.3	9.2	0.2	0.2	0.1
10.0	14.2	10.1	0.2	0.2	0.1
10.0	14.2	10.1	0.4	0.4	0.5
10.0	14.3	10.2	0.4	0.4	0.5
None	4.2	None	0.4	0.4	0.5

TABLE V  
RECOVERIES OF ARSENIC AND BISMUTH FROM WATERY SOLUTION IN PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG	MG	MG
None	3.6	None	None	None	0.5
None	3.6	None	None	None	0.5
10.0	13.5	9.9	None	None	0.5
10.0	13.7	10.1	None	None	0.5
None	3.6	None	None	None	10.0
20.0	23.0	19.4	None	None	20.0

TABLE VI  
RECOVERIES OF ARSENIC AND BISMUTH FROM BLOOD IN PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG	MG	MG
None	5.8*	None	None	Faint trace (?)	20.0
None	5.8*	None	None	Faint trace (?)	20.0
20.0	26.1*	20.2	None	Faint trace (?)	20.0
20.0	25.2*	19.3	None	None	20.0

The separation and quantitative determination of bismuth and arsenic were carried out according to the above described method with specimens of human blood, ox blood, and with water containing known amounts of arsenic and bismuth.

TABLE VII

RECOVERIES OF ARSENIC AND BISMUTH FROM WATER IN PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG	MG	MG
None	5.5*	None	None	None	10.0
None	5.3*	None	None	None	10.0
20.0	25.1*	19.7	2.0	2.1	20.0
20.0	26.0*	20.1	2.0	2.8	20.0
None	5.9*	None	None	Faint trace (?)	20.0

It seemed advisable also to study the influence of the presence of antimony upon the recoveries of arsenic and bismuth. Antimony trichloride was added to some of the blood specimens and also to the watery solutions of arsenic and bismuth compounds in order to investigate the influence of antimony upon the separation and determination of arsenic and bismuth.

## SUMMARY

1 The method suggested in this paper permits the separation and quantitative determination of both bismuth and arsenic in the same biologic specimen (blood).

2 The presence of antimony in reasonable amounts does not interfere with the determination of arsenic in the distillation products, nor with the determination of bismuth in the residue according to Leonard's method.

3 The amounts of salts resulting from the distillation process is not large enough to interfere with the determination of bismuth in the residue after the volatilization of the arsenic trichloride.

4 It is necessary to carry out blank determinations simultaneously with every new batch of reagents used, since the amounts of arsenic present in the reagents varies.

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## A CLINICAL MODEL OF THE HADEN-HAUSSE HEMOGLOBINOMETER\*

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IN 1930<sup>1</sup> I described a new hemoglobinometer with which hemoglobin estimations can be made simply and accurately. This instrument has proved most satisfactory but its distribution is necessarily limited by reason of its initial cost. It is the hemoglobinometer of choice if available. Recently a much less expensive hemoglobinometer has been devised, which utilizes the principle employed in the larger instrument, and is sufficiently accurate for routine clinical work.

*Description*—The principal components of the instrument are illustrated in Fig. 1, the parts of the capillary comparison cell in Fig. 2, and the instrument assembled for use in Fig. 3.

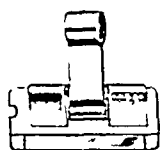
A wedge shaped chamber to hold the diluted blood is made in the capillary comparison cell, *D* (Fig. 1) by the combination of glass wedge, *A* (Fig. 2) and plane glass plate, *C* (Fig. 2), if the hemoglobin is within the 8 to 18 gram range or by glass wedges *A* and *B*, which doubles the depth of the solution, if the hemoglobin is below 8 grams per hundred cubic centimeters. The other essential parts of the instrument are a comparator with a series of rectangles, the lower half of which is the color standard complement and the upper half of which is the acid hematin complement when the filled comparison cell is in place for reading. The cell *D*, and the comparator *F*, together take the place of the comparison slide used in the laboratory model of the Haden-Hausser hemoglobinometer. A magnifier with a track on which the comparator slides, is added to facilitate reading. Light filters for both daylight (Fig. 1, *G*) and artificial light (Mazda bulb) (Fig. 1, *H*) complete the instrument.

*Method of Use*—The blood is diluted 1:10 with tenth-normal hydrochloric acid in a Thoma white cell pipette and the capillary emptied immediately. After standing for thirty minutes, the wedge shaped chamber of the comparison cell is filled, and the cell placed in the sliding comparator. The sliding comparator carrying the comparison cell and light filter is now suspended on the slide track of the magnifier. On viewing the comparator slide through the magnifier several rectangles are seen. The lower color standard complement is the same throughout as the glass is of uniform thickness, the acid hematin complement is different in each rectangle since the diluted blood is in a wedge-shaped chamber. The comparator is then moved from right to left across the field of view until a close match is found between the upper or acid hematin complement and the lower or color standard complement of one of the illumi-

\*From the Cleveland Clinic.

This instrument was developed and is now being made by C. A. Hausser and Sons and is sold by A. H. Thomas Company, West Washington Square, Philadelphia.

nated rectangles. This rectangle is next brought into the center of the field. The upper half of the rectangle to the left is then a darker tint and of that to the right a lighter tint if the match is correctly made.



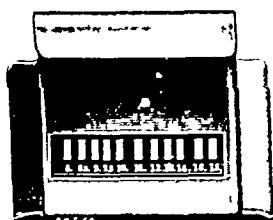
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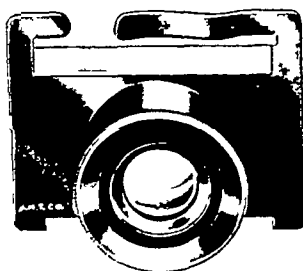
G



H

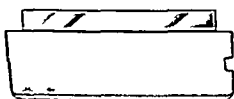


F

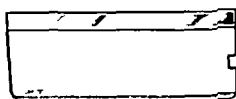


J

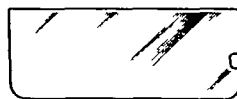
Fig. 1—Principal components of the hemoglobinometer. D, Capillary comparison cell held in spring clip. F, Sliding comparator with hemoglobin-gram scale. This carries both the comparison scale and the light filter. G, Light filter for use with daylight. H, Light filter for use with Mazda lamp. J, Magnifier with support and slide track to carry comparator F.



A

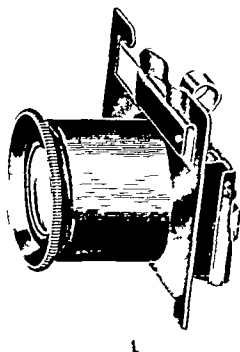


B

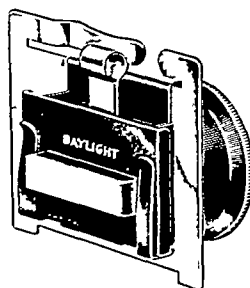


C

Fig. 2—Components of capillary comparison cell. A, glass wedge. B, glass wedge. C, plane glass plate.



A



B

Fig. 3—Hemoglobinometer assembled for use. A, front view. B, rear view.

The numerical value in grams of hemoglobin per hundred cubic centimeters of blood is indicated beneath the matched rectangle. If the hemoglobin is too low to read, a capillary cell made with wedges A and B instead

of *A* and *C* is filled with the diluted blood remaining in the pipette. The reading is then made and the value divided by 2 to obtain the hemoglobin in grams per hundred cubic centimeters.

*Experimental Observations*—In calibrating this instrument, I have made comparative readings with the oxygen capacity method of Van Slyke, the non content method of Wong, and with values obtained with the laboratory model of the Haden-Hausser hemoglobinometer. These results are shown in Table I. It is apparent that readings well within the needs of clinical work can be made with this instrument. The readings can be made at the bedside, if desired. The cost of the instrument is small and the standard is permanent.

TABLE I

COMPARISON OF HEMOGLOBIN READINGS WITH CLINICAL MODEL OF THE HADEN HAUSSER HEMOGLOBINOMETER AND OTHER METHODS

SPECIMEN NO	CLINICAL MODEL HADEN HAUSSER HEMOGLOBIN OMETER	LABORATORY MODEL HADEN HAUSSER HEMOGLOBIN OMETER	OXYGEN CAPACITY METHOD VAN SLAKE	IRON CONTENT METHOD NEW WONG
	Gm per 100 cc	Gm	Gm	Gm
1	80	80	79	77
2	90	90	86	85
3	100	100	97	95
4	115	115	112	109
5	130	130	130	128
6	140	140	143	140
7	150	155	157	154
Mean	115	116	115	113

*Discussion*—The instrument is calibrated in grams only, using the two standard methods of calibration, namely, the oxygen capacity and non content methods. The readings obtained with the hemoglobinometer can be compared then with those obtained by the two chemical methods. This is the first requisite in all hemoglobin estimations. The second problem concerns the translation of values so obtained into percentage terms. I have discussed the question of hemoglobin standards recently elsewhere<sup>2</sup> and have offered a

TABLE II

SPECIMEN NO	RED CELL COUNT IN MILLIONS PER CM	HEMOGLOBIN IN GRAMS PER 100 CC WITH CLINICAL MODEL, HADEN HAUSSER HEMOGLOBINOMETER
	Millions	Grams
1	5.02	16.0
2	4.00	12.5
3	4.65	15.0
4	5.50	17.0
5	4.78	15.0
6	4.57	14.0
7	4.25	13.5
8	5.31	16.0
9	5.80	17.0
10	4.92	15.0
Mean	4.88	15.1

simple solution of the confusing problem of choosing between standards suggested by different workers in this field. It is a very easy matter for each laboratory worker using the hemoglobinometer to determine for himself the standard to use. The most important point is the use of a standard which in the hands of the worker employing it gives a color index of  $1.00 \pm 0.10$ . This is done by making hemoglobin determinations and accurate red cell counts simultaneously on each of about ten normal bloods. The number of grams of hemoglobin per hundred cubic centimeters per five million cells, or the hemoglobin coefficient is calculated from these data, and the figure so obtained taken as 100 per cent in figuring results in percentage terms. This is illustrated in Table II.

Since the average normal blood in the example given with a red cell count of 4,880,000 per cubic centimeter contains 15.1 grams of hemoglobin per hundred cubic centimeters, it is apparent that the hemoglobin content would be 15.4 grams if the count were 5,000,000 red cells. The figure so obtained (15.4 grams) is thus taken as 100 per cent. When so determined, the color index in normals should always be  $1.00 \pm 0.10$ . In reporting results in percentage obtained in this manner, confusion is prevented by indicating that 100 per cent equals the hemoglobin in grams per hundred cubic centimeters per five million cells in normal persons. The actual number of grams per hundred cubic centimeters makes no difference if the color index in normal persons is 1.00 within the limits of error.

*Summary*—A new inexpensive hemoglobinometer is described which employs (1) the acid hematin principle of hemoglobin determination, (2) a permanent standard, (3) a very low dilution ratio (1 to 10) in a standard white cell pipette, (4) reads directly in grams, and (5) is calibrated by the oxygen capacity and iron content methods.

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## A NOTE ON THE PRESERVATION OF ACID-FAST BACTERIA IN VACUO\*

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IT HAS been the usual practice in this laboratory to maintain the stock of mycobacteria by subculture every four to six weeks. This procedure, although effective has the following disadvantages (1) if, as may occasionally happen, too long a period is allowed to elapse between transfers, i.e., eight weeks or longer, some strains may have died out and been lost, (2) considerable time is used for this purely routine work, (3) the necessary media are relatively expensive and troublesome to prepare.

It was thought that the method of Brown for preserving the common pathogens and saprophytes in vacuo might be adapted to mycobacteria and afford a simple means of maintenance for long periods without transfer. Brown's method in brief is as follows: a number of small pieces of filter paper about  $\frac{1}{4}$  by  $\frac{1}{2}$  inch are placed in small glass vials ( $1\frac{1}{2}$  inches by  $\frac{3}{8}$  inch) plugged with cotton and sterilized. Using a vial for each strain a loopful of a young culture is placed on each bit of paper in the vial. A number of vials with various strains of bacteria are placed in half-pint or pint bottles over calcium chloride. A small manometer is put within the bottle so it can be read from outside. A little vaseline is placed around the lip of the bottle and a special metal cap is lightly placed on top. The bottle is then evacuated under a bell jar with the use of a good vacuum pump for fifteen or twenty minutes until a high vacuum is obtained. An is allowed to enter the bell jar suddenly which forces the cap down tightly on the bottle. The bottle is then placed in a hand crimping machine and the cap crimped. It is then stored in the ice box. (The bottle caps and crimping machine may be obtained from the Crown, Cork and Seal Company of Baltimore.)

To preserve acid-fast the above procedure was modified slightly. The vials were plugged and sterilized empty. With a sterile platinum spatula kernels of the hard wrinkled growth were transferred to the vials, the vials placed in a bottle, and the bottle evacuated and crimped as above described and left in the refrigerator for eleven months. Four-week-old cultures from Petrioff and Lubenau medium slants were used. Thirty-one strains, each strain in one vial, were stored in this way. They are shown in Table I.

After eleven months the bottle was opened and the hard dry "kernels" were easily broken up with a spatula and a little was transferred to slants of Lubenau, Petrioff, Sweeny cream medium and also Petriagnani medium in the case of pathogens and glycerin agar slants in the case of the saprophytes.

\*From the Department of Bacteriology The Johns Hopkins School of Hygiene and Public Health

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TABLE I  
MYCOBACTERIUM TUBERCULOSIS

HUMAN	BOVINE	AVIAN	SAPIROPHYTES
Sarumac H 37	B 1	BA1 Parrot	B L S
Lov 7	Princeton 32	BA1 Pheasant	Timothy 6292
Lov 9	Sarumac B 1	BA1 Swine	Sarumac Timothy
Lov 62	Park 613	Vin Es H3701	Timothy A M 213
Lov 71	Zinsner B 1	Vin Es stock	Butter R
	Opie B 1	Vin Ls 4957	B moelleri
	Hvg Lab 110	Vin Es 3732	Red timothy
		Tizzer A 3	B smegmatis
			Sarumac mist
			Sarumac "lepra B"
			B leprae Am Museum
			"Apr "

In forty-eight hours all of the saprophytes had grown out. In three weeks most of the pathogens showed some growth and in four weeks all of the pathogens showed excellent growth. Of the media used the Petriagnai malachite green medium appeared to give the finest growth. Three of the pathogenic strains were evidently contaminated in the vials, for the contaminants as well as the mycobacteria grew out on all cultures. Two of the three contaminated were easily purified by fishing isolated colonies from the Petriagnai slants. This was possible because the malachite green dye in the medium inhibited the contaminant while allowing the acid-fast to grow. In the case of the third contaminated strain acid digestion methods had to be resorted to, to purify the culture.

About 1 mg each of the preserved human strain H 37 and of the bovine strain Princeton 32 was injected into two guinea pigs. The human strain was first injected subcutaneously in the right groin and seven weeks later intraperitoneally. The autopsy was made eleven weeks after the first injection and showed a general glandular enlargement with caseation, a rolled up thickened, caseous omentum, an enlarged granular spleen and implantations on the surface of the liver and the parietes. The bovine strain was inoculated intraperitoneally four weeks before autopsy. The parietes were roughened with innumerable minute translucent tubercles, the spleen was smooth and a little enlarged, the omentum rolled up and caseous, the glands somewhat enlarged and a few very small necroses in the liver. It appears from our results that acid-fast bacteria can be preserved for at least eleven months by the method described without any apparent loss of virulence.

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|-----------------|---|
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# A METHOD FOR THE COLORIMETRIC DETERMINATION OF THE URINARY CHOLESTEROL<sup>†</sup>

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A FEW attempts have been made to determine the amount of cholesterol present in the urine. All methods for the estimation of urinary cholesterol necessitate two steps: the first is complete extraction of the urine with a lipid solvent, and the second is the determination of the cholesterol content of the extract thus obtained. The extraction procedures generally employed are either of the following: the urine is directly extracted by lipid solvents or it is first evaporated to dryness and then extracted. The former is a lengthy process requiring repeated extraction and separation of a large sample of urine over a protracted period of time. The latter is cumbersome because the evaporation of a large volume of urine and the complete extraction of the resultant gummy residue is both tedious and difficult. We have been unable to obtain satisfactory extractions of the urine by either of these methods.

Two means for the determination of the cholesterol content of the urinary extract based on the gravimetric and the colorimetric principles have been applied. Gardner and Gainsborough<sup>1</sup> extracted large volumes of alkalized urine with ether for five to six days. The ethereal extract thus obtained was saponified, and the cholesterol measured by the digitonide method. The extracted urine was further hydrolyzed with glacial acetic acid. The resultant solution was extracted with ether and the cholesterol content again estimated. They thus found, after acid hydrolysis, what they believed to be an "ethereal sulphate" of cholesterol. The gravimetric method (Windaus<sup>2</sup>) is adequate since the relationship between free cholesterol and its digitonide has been well established. Nevertheless, the difficulties inherent in the performance of a gravimetric determination make this process so complicated that it is not suitable for routine use.

Criticism of the colorimetric method as applied to urine has centered mainly on the pigmentation and occasional turbidity of the extracted solution which renders the final color brownish or yellowish. We have succeeded in obviating this difficulty by the introduction of a procedure which extracts the lipoids while it does not extract the pigments. Furthermore, our method requires only a relatively small amount of urine for each determination, it can be carried out in from two to three hours and is accurate as far as can be judged from estimations of duplicate samples.

The principle of the method is based chiefly on the observation of de Toni<sup>3</sup> who found that the tungstic acid precipitate of blood contains all the chole-

\*From the Department of Medicine, New York Post-Graduate Medical School and Hospital.  
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terol We have repeatedly confirmed this observation Gardner and Gainsborough studied the urine from a case of "subacute parenchymatous nephritis", they acidified the urine with acetic acid and boiled it, the resultant solution was filtered and the precipitate was washed and dissolved in 2 per cent caustic soda, this was then extracted with ether and the cholesterol determined The filtrate was alkalinized and repeatedly extracted with ether, the thoroughly extracted filtrate was then evaporated to dryness, the residue hydrolyzed with glacial acetic acid, extracted and saponified They thus found that 91 per cent of the free and ester cholesterol apart from the "ethereal sulphate" was precipitated along with the proteins In the method described below all the urinary cholesterol is precipitated with a tungstic acid precipitate We have found no evidence for the existence of an "ethereal sulphate" of cholesterol

#### METHOD

*Total Cholesterol*—The urine must be neutral or slightly acid in reaction If alkaline, it should be neutralized with dilute acetic acid One hundred cubic centimeters of the urine are measured into a centrifuge bottle of 150 c c capacity To this are added 5 c c of a 50 per cent solution of sodium tungstate\* and 5 c c of a 10/3 N solution of sulphuric acid, the sulphuric acid being added drop by drop The bottle is then centrifuged for five to ten minutes and the supernatant fluid discarded About 150 c c of boiling distilled water are added to the precipitate which is broken up with a glass rod and subsequently centrifuged The supernatant fluid is discarded This is repeated, so that altogether the precipitate is washed two or three times with boiling distilled water Fifty cubic centimeters of an alcohol ether mixture (3 to 1) are added to the precipitate in the bottle This is placed on an electric plate and allowed to boil for about one minute While hot, the extract is filtered through a fritted glass filter of fine porosity into a small clean Erlenmeyer suction flask and slight suction is applied The precipitate is washed once with 25 c c of hot alcohol ether mixture and twice with 15 c c of boiling absolute alcohol The filtrate in the flask is transferred to a beaker and evaporated to dryness over a hot plate If the original precipitate has been adequately washed with boiling distilled water, the brownish residue will just cover the bottom of the beaker Fifteen cubic centimeters of petroleum ether (B P 30° to 50° C) are added to the beaker and evaporated to approximately half the original volume on an electric plate (Avoid open flames) The petroleum ether extract is decanted carefully into a 20 c c volumetric flask The petroleum ether extraction and the subsequent addition to the flask is repeated two or three times The combined extract in the 20 c c volumetric flask is evaporated to dryness over a steam-bath or in an incubator To the residue are added 20 c c of chloroform Five cubic centimeters of the chloroform extract are transferred to a test tube To another test tube are transferred 5 c c of a standard solution prepared by dissolving 10 mg of cholesterol in 100 c c of chloroform The second test tube with the

\*The sodium tungstate solution should be approximately neutral in reaction

standard solution, therefore, contains 0.5 mg of cholesterol. To each of these are added 2 cc of acetic anhydride and 0.1 cc of concentrated sulphuric acid. The tubes are placed in a dark closet at room temperature for five minutes, then in a refrigerator for another ten minutes.<sup>4</sup> A comparison is made between the green colors of the standard and unknown solutions. If the color of the unknown deviates by more than 35 per cent to 40 per cent from that of the standard solution a suitable aliquot of the chloroform extract must be used. As an alternative, several standard solutions may be used. A 20 mg per cent standard, a 10 mg per cent standard, and a 5 mg per cent standard may be prepared and the appropriate one employed. We have found the former procedure quite convenient. The amount of total cholesterol in the urine is then calculated by applying the following formula:

$$\frac{R_s}{R_u} \times \text{mg cholesterol in standard} \times \frac{100}{A} = \text{mg cholesterol per 100 cc of urine}$$

Where  $R_s$  = the reading of the standard solution,  $R_u$  = the reading of the unknown solution, and  $A$  = the amount of urine represented by the chloroform aliquot

*Free and Combined Cholesterol*—After the total urinary cholesterol has been determined, twice the amount of chloroform extract that was necessary for that determination is evaporated to dryness and taken up in about 20 cc of an alcohol ether (3 to 1) mixture. The free and combined cholesterol fractions are estimated by fixing the free cholesterol in the form of a digitonide and determining the esters colorimetrically as is done for blood.

The procedure just described is suitable for urines rich in cholesterol as in certain cases of nephritis and other pathologic conditions. For normal subjects, a larger amount of urine should be extracted, at least 400 to 500 cc of urine must be taken for each determination. This is accomplished by combining the petroleum ether extracts from four or five 100 cc samples treated as outlined and determining then cholesterol content. The variations in normal and pathologic urines will be discussed in a subsequent paper.

#### DISCUSSION

The tungstic acid filtrates of urines, from cases often designated as lipoid nephrosis, were evaporated to dryness and extracted very carefully with alcohol ether (3 to 1). Since cholesterol was never found in the filtrates thus studied, it is assumed that all the cholesterol is brought down with the tungstic acid precipitate. However, Gardner and Gamsborough have reported the presence of an "ethereal sulphate" of cholesterol in the filtrate of a deproteinized urine from a case of "sub-acute parenchymatous nephritis." They studied only one sample of urine but we have been unable to confirm this observation in four "nephrotic" urines. The filtrates from such urines were hydrolyzed with glacial acetic acid by boiling under a reflux condenser for five hours. The resultant solution was evaporated and the residue thoroughly extracted with an alcohol ether mixture. No trace of cholesterol was found in these extracts by the colorimetric method.

The water washes from the precipitate were studied in a manner similar to the urine filtrates, and again no cholesterol was found. Thorough washing

TABLE I

DETERMINATIONS OF THE CHOLESTEROL CONTENT OF NORMAL AND PATHOLOGIC URINES\*

URINE	FIRST SAMPLE			SECOND SAMPLE			AVERAGE PER CENT DEVIATION FROM MEAN			REMARKS
	TOTAL CHOL MG %	ESTER CHOL MG %	FREE CHOL MG %	TOTAL CHOL MG %	ESTER CHOL MG %	FREE CHOL MG %	TOTAL CHOL	ESTER CHOL	FREE CHOL	
1	4.52	3.35	1.17	4.57	3.31	1.25	0.6	0.4	3.5	Chronic diffuse glomeru- lar nephritis (with nephrotic component)
2	2.10	1.38	0.72	2.05	1.31	0.74	1.0	2.1	1.5	Chronic diffuse glomeru- lar nephritis
3	2.00	1.23	0.77	1.97	1.08	0.89	0.7	6.5	7.1	Chronic diffuse glomeru- lar nephritis
4	2.04	0.96	1.08	2.16	1.08	1.08	2.8	5.8	0	Chronic diffuse glomeru- lar nephritis
5	3.00	1.41	1.59	3.03	1.53	1.5	0.5	3.9	3.2	Chronic diffuse glomeru- lar nephritis (with nephrotic component)
6	0.04			0.04			0			Normal
7	4.47			4.69			2.3			Chronic diffuse glomeru- lar nephritis (with nephrotic component)
8	0.31			0.29			4.0			Normal
9	1.19			1.19			0			Diabetes mellitus
10	0.88			0.87			0.4			Obstructive jaundice
Average							1.2	3.5	3.1	

\*The above figures are no indication of the total urinary cholesterol since the urine volume output is not considered

of the tungstic acid precipitate allows for better extraction with the alcohol ether mixture. For example, four 100 cc samples of urine from a case of "lipoid nephrosis" were precipitated with tungstic acid. In two of these, the precipitate was thoroughly washed with boiling distilled water, the other two were not washed. The extract from the washed precipitates contained 4.47 mg per cent and 4.69 mg per cent of total cholesterol respectively. Those from the unwashed precipitates contained 2.75 mg per cent and 3.66 mg per cent respectively.

The extraction of the precipitate twice with the alcohol ether mixture and twice with hot alcohol is adequate. The subsequent extraction of the alcohol ether residue with petroleum ether is also complete as is evidenced by duplicate determinations (Table I).

## SUMMARY

A comparatively rapid colorimetric method for the determination of urinary cholesterol in normal and pathologic urines is described.

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## A CONTINUOUS EXTRACTION APPARATUS\*†

S E OWEN, PH D, AND W L OLSEN, B S, SALT LAKE CITY, UTAH

ONE phase of our work on pancreatic function tests required the extraction of urine with butyl alcohol. To eliminate considerable time consuming labor with separatory funnels, the apparatus herein described was devised for the particular purpose of extracting relatively small quantities of urine. We believe that it is simpler in construction than most apparatus, and is more satisfactory for our purpose than any other with which we are familiar.

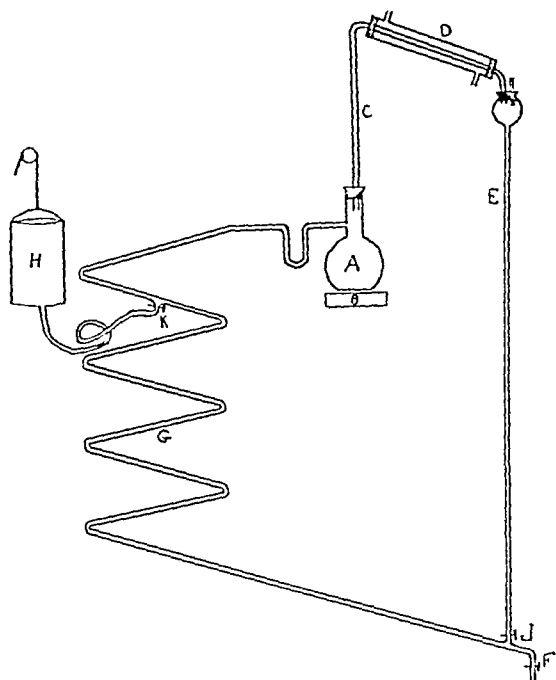


Fig. 1

The advantages are as follows: (1) It may be used in the extraction of either a stationary column of liquid or a continuously flowing column. (2) Small quantities of a substance may be as conveniently extracted, as larger quantities. (3) Only a small amount of solvent is required. (4) The small bore of the extraction tube renders extraction more thorough and rapid, as do the sharp bends which tend to break the droplets of solvent into smaller droplets. The latter property could be improved by either indenting the tube

\*From the Department of Pharmacology and Physiology, School of Medicine, University of Utah.

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†Research assistance provided by grant of American Association for the Advancement of Science.

at frequent intervals, or by inserting a fine coiled noncorrosive wire into the extraction tube (5) By modifying the apparatus to allow a lowering of the distilling flask and a change in the points of entrance of the solvent and the liquid to be extracted, it could be used for extraction with solvents heavier than the liquid to be extracted (6) The apparatus is simple to construct and the materials required may be found in almost any laboratory

*Construction*—The extraction column (*G*) is made of 8 mm bore Pyrex or soft glass. This column is given a zigzag shape, the slope being about four inches per foot. While our apparatus rarely had more than four such sections, the only limiting factor to the length of the extraction column would be the room height. The solvent column (*E*) consists of a 4 mm bore glass tube welded to the larger column. The urine inlet is situated at (*K*) near the top of the zigzag column. Tube (*E*) which carries the butyl alcohol from the condenser must be of sufficient length to allow the butyl alcohol column to overbalance the column of urine and superimposed butyl alcohol in tube (*G*) after calculating the lengths of the liquid columns required with reference to the specific gravities of the substances employed, it is well to allow one or two inches more on the butyl alcohol column (*E*). The reservoir for urine (*H*) is placed on an adjustable stand or arranged with a pulley and cord suspension as shown, thus allowing adjustment of the urine level when the apparatus is used for continuous flow extraction.

*Use*—In use, the flask (*A*) has placed in it about 100 cc of the solvent, and tube (*E*) is filled with the solvent. The material to be extracted is then introduced into the reservoir and the extraction column (*G*) is filled. The stopcock at (*J*) is then opened and extraction commences when the butyl alcohol distills over into the small receiver at the top of tube (*E*). Heat regulation of the hot-plate (*B*) must be adapted to the individual apparatus, we find that when the returning drops of solvent are spaced about two inches apart, the apparatus functions satisfactorily. When a continuous flow is desired, the stopcock at (*F*) is opened allowing a slow stream of urine to flow out. To prevent excessive refluxing in the vapor column (*C*), this column is insulated with asbestos rope.

We believe that this apparatus could also be used to advantage in the recovery of drugs from body fluids, the extraction of tissue juices and extractions, and numerous other similar operations.

# A SIMPLE METHOD OF REMOVING MUSCULAR TREMOR WAVES FROM THE ELECTROCARDIOGRAM\*

C S DANZER, M D , F A C P , BROOKLYN, N Y

THE electrocardiogram is admittedly becoming an increasingly indispensable procedure in the diagnosis and prognosis of many heart disorders. It is often sufficiently typical to be diagnostic of several heart conditions

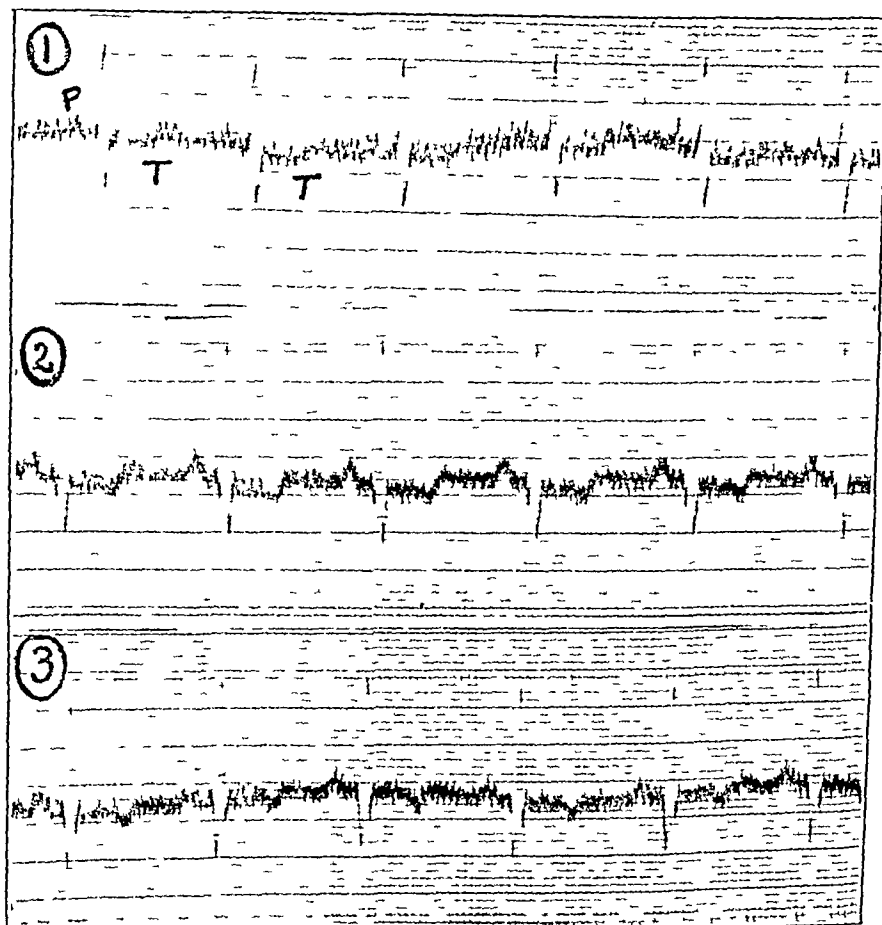


Fig 1—Electrocardiogram taken in the usual manner

Despite adequate care in the taking of the tracing its value is often reduced or lost because the addition of wavelets due to muscular tremors make interpretation difficult

\*From the Cumberland Hospital and Brooklyn Cancer Institute of the City of New York  
Received for Publication September 20 1932



In some cases sufficient rest in the recumbent position before taking the electrocardiogram reduces these, in others this is insufficient. For such cases I have developed a method which is generally successful in removing these wavelets. Its description is the object of the present communication.

The method consists briefly of the administration of 8 cc of the elixir of diallyl barbituric acid (Dial) and allowing the patient to lie down for

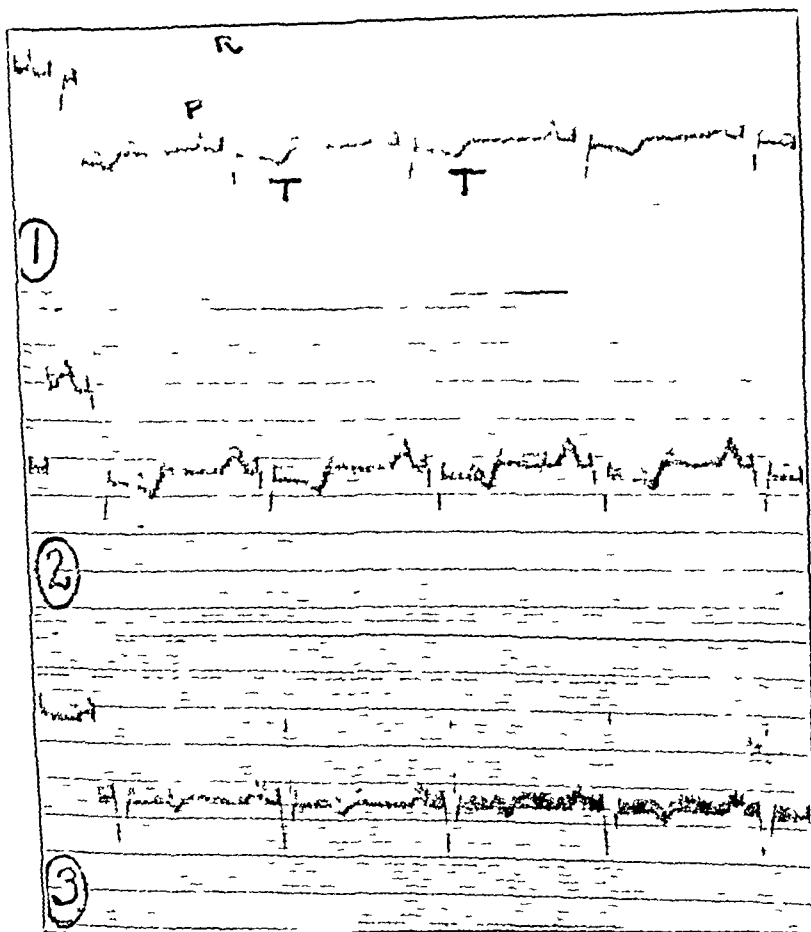


Fig. 2—Electrocardiogram from same patient after Dial and twenty minutes rest.

twenty or thirty minutes before the electrocardiogram is taken. It is probable that other sedatives may act similarly.

Figs 1 and 2 were taken before and after the use of the sedative, and are self explanatory. It may be seen that in Lead 1 the negative T-wave which is such an important finding, has been completely obliterated by the disturbing muscular vibrations.

## AN INEXPENSIVE BUT DURABLE METABOLIC CAGE FOR SMALL ANIMALS\*

A RICHARD BLISS, JR, PHM D, M D, MEMPHIS, TENN

THE usual cost of manufacturers' animal cages, which are large enough and strong enough for use in metabolic studies with small animals, such as cats, adult rabbits, and small dogs, is prohibitive for moderate college laboratory budgets, especially in the event a fairly large number of cages is needed. The cage

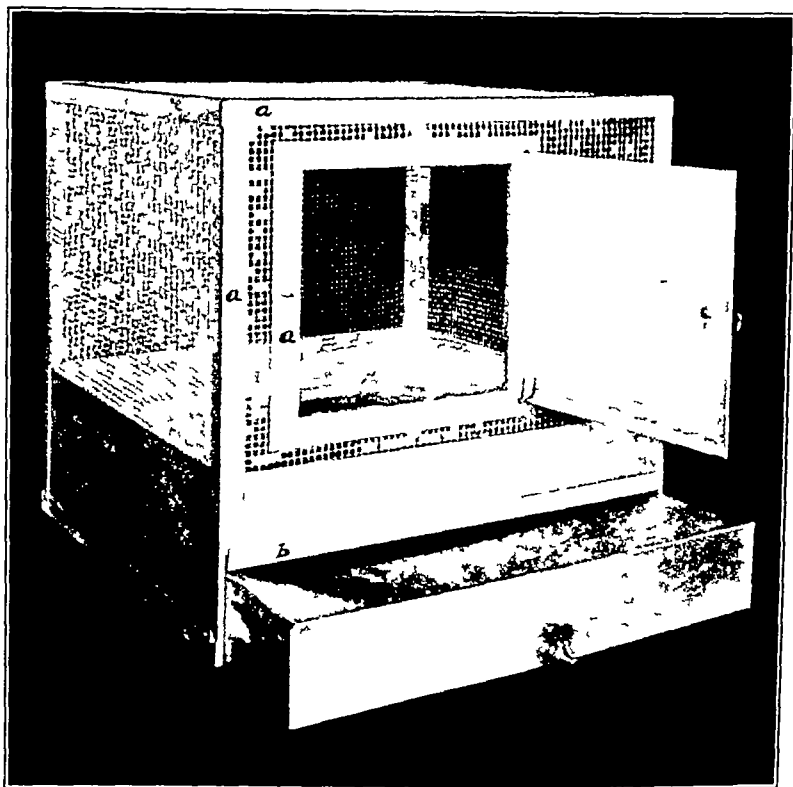


Fig 1—An inexpensive durable cage for small animals made in the University shop

shown in Fig 1 was devised in the Division of Pharmacology of the University of Tennessee with the collaboration of Mr. M. B. Parker, B. S., in charge of the "University Apparatus Company," which is housed in the Anatomy Building of this institution.

The cage is 18 inches by 18 inches by 18 inches, and is made of galvanized iron (22 gage) and hardware cloth (4 by 4), the parts soldered together. The edges

\*From the Division of Pharmacology, College of Medicine, University of Tennessee.  
Received for publication August 24, 1932.

(a) of the cage are made of bent strips of the galvanized iron (see Fig 1) The outside depth of the pan is  $3\frac{1}{2}$  inches, the bottom of the pan, one piece of galvanized iron, is sloped downward toward the stopcock the only seam running from the stopcock to the top of the drawer front The removable sliding bottom (b), supported immediately above the pan is made of 8 by 8 hardware cloth, edged with galvanized iron the outer edge bent upward as shown in the illustration An ordinary stopcock and a hook for the hinged door complete the cage The total weight is 18 pounds which permits ready stacking and transfer The cage is painted inside and out with aluminum paint The total cost including materials and mechanic's charge is \$8.50 per cage Twenty-four of these cages have been in constant use for over a year and have proved quite satisfactory and durable

## Item

### The American Society of Clinical Pathologists

The American Society of Clinical Pathologists held their twelfth annual convention in Milwaukee from June 9 to 12 Dr A G Foord of Pasadena, California, was inducted into office as President for the years 1933 and 1934 The officers elected are as follows

President elect Dr F H Lamb, Davenport, Iowa  
Vice President Dr J J Seelman, Milwaukee, Wisconsin  
Secretary-Treasurer Dr A S Giordano, South Bend, Indiana

The Ward Burdick award was given to Dr A H Sanford of Rochester, Minnesota

The first prize for Scientific Exhibits was given to a group of four men made up of Drs A O Gettler, H S Martland, A V St George, and Charles Norris Second prize was given to Dr W D Stovall of Madison, Wisconsin

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**LEUKOCYTES**, Schilling Blood Count in the Pneumonias of Infancy and of Childhood, Rogatz, J. L. *Am J Dis Child* 45: 1022, 1933

Fifty cases of juvenile, bronchial and lobar pneumonia were studied with reference to changes occurring in the leukocytes during these infections. The Schilling classification of immature cells was used. The daily changes in these elements of the blood were observed and are described in detail. It was possible to anticipate lysis in bronchial pneumonia or the occurrence of the crisis in the lobar types by many hours. Descriptions, with charts and hemograms, are given of striking cases, which illustrate this observation.

From these and other studies it is concluded that the Schilling blood count is of greater value than the ordinary smear in revealing changes in the patient's condition, that it reflects these changes more accurately and somewhat in advance of their clinical appearance and that it should therefore replace the usual differential examination of the blood in routine practice.

**LEUKOCYTES**, Cytoplasmic Changes in Infection, Sutro, C. J. *Arch Int Med* 51: 747, 1933

The author reports a study of the presence or absence of "toxic granules" in the circulating leukocytes in 200 cases of various infections and determined the "degenerative index" by the formula

$$\frac{\text{Toxic polymorphonuclear cells}}{\text{Total polymorphonuclear cells}} = \text{index}$$

$1 = 1 \text{ plus}, \frac{3}{4} = 3 \text{ plus}, \frac{1}{2} = 2 \text{ plus}, \frac{1}{4} = 1 \text{ plus}$

In a study of two hundred cases in which the blood was investigated for toxic granules, it was found that the continued presence of a four plus degenerative index would indicate a serious prognosis. A four plus degenerative index in cases in which bacteremia or pyemia is suspected points to generalized infection in spite of a negative blood culture. In local infections (mastoiditis, acute appendicitis, etc.) without complications, there will be no toxic granules, or at most the degenerative index will be one or two plus. However, should these conditions be complicated by bacteremia or peritonitis, toxic granules appear in many or all of the polymorphonuclear cells.

**BILIARY CRYSTALS**, Diagnostic Importance of, Rofsky, H. A. *Am J M Sc* 185: 851, 1933

Various types of biliary crystals found in preoperative specimens of bile were compared with the crystalline elements found in the scrapings of calculi or bile removed from the gall bladder at the time of operation.

In 91.3 per cent of the cases the presence of these crystals in the bile, preoperatively, indicated calculous or noncalculous cholecystitis, irrespective of the history or radiographic findings.

Bile microscopy should be employed more frequently, especially in patients with a more or less persistent gastric history.

**BLOOD Determination of Iron Content of in Children, Sobel, P I., and Drechter, I J**  
*Am J Dis Child* 45 186, 1933

**Reagents**—Standard Solution A Dissolve 0.702 gm of ferrous ammonium sulphate (Merck's reagent) in about 50 cc of water, add 20 cc of 10 per cent iron free sulphuric acid, warm and add tenth normal potassium permanganate solution until a faint pink color persists. Dilute to 1,000 cc. One cubic centimeter of this solution will contain 0.1 mg of iron.

Standard Solution B Dilute standard solution A with an equal volume of water. One cubic centimeter of this solution will contain 0.05 mg of iron.

**Sulphocyanic Acid** Dissolve 100 gm of ammonium thiocyanate in 100 cc of 65 per cent (by weight) sulphuric acid. Measure the volume, transfer to a large separatory funnel and shake the mixture with three fourths of its volume of amyl alcohol. Let the solution settle and discard the aqueous solution. Add an equal volume of water, and shake thoroughly. Preserve the water extract. Repeat the extraction of water, and combine the two extracts, which contain about 7 per cent of sulphocyanic acid. Saturate the water with solid mercuric sulphocyanate so that the excess remains on the bottom of the container. Allow to stand overnight in a dark bottle.

**Persulphate Solution** This is a saturated solution of potassium persulphate free from iron in distilled water.

**Potassium Chlorate Solution** Dissolve 10 gm of chemically pure potassium chlorate (iron free) in 100 cc of water.

**Extraction Mixture** Combine equal parts of ethylene glycol monobutyl ether and ethyl ether. The ether does not have to be of the highest purity, as the presence of mercuric sulphocyanate prevents the oxidation of sulphocyanic acid by peroxides which may be present in the ether.

**Technic** One cubic centimeter of oxalated blood is diluted with 4 cc of iron free distilled water. Using the same pipet with which the blood was measured, transfer 1 cc of the diluted blood into a pyrex test tube (25 by 20 cm). And 1 cc of concentrated sulphuric acid (free from iron), heat the tube until white fumes are given off, cover it with a small watch glass, and continue heating for two minutes. Remove the flame, place the tube at an angle of 45 degrees, and add 1 cc of 10 per cent potassium chlorate carefully, drop by drop. Place the tube in an upright position and heat until white fumes are evolved. Again place the tube at a 45 degree angle, add an additional 0.3 cc of the potassium chlorate solution, and boil the mixture until all the chlorine is removed. Allow the tube to cool, dilute the contents with 15 cc of water, and add a drop of persulphate solution. Add exactly 25 cc of the extraction mixture, followed by 5 cc of the sulphocyanic acid reagent. Fit the tube with a rubber stopper and shake thoroughly. The solution separates into two distinct layers, the upper of which is red and the lower colorless. The red color is compared in the colorimeter with a suitable standard solution of iron which has been digested and treated in the same manner as the unknown. Two standards are prepared as described under "Reagents."

**Calculation** The following formulas are used in the colorimetric determinations

$$\frac{R_s}{R_x} \times 500 = \text{milligrams of iron per hundred cubic centimeters of blood}$$

S = Strength of the standard      R<sub>s</sub> = reading of the standard

R<sub>x</sub> = reading of the unknown

The iron content of the venous blood of 48 relatively normal children ranged from 10 to 52 mg per hundred cubic centimeters. The average was 43.9 mg.

In 57 children with various pathologic conditions, the blood iron content ranged from 18.2 to 74 gm per hundred cubic centimeters.

No constant relationship was found between the amount of hemoglobin, determined by the Sahli and Newcomer methods, and the blood iron concentration. In 88 per cent of 135 cases the amount of hemoglobin determined by the Newcomer method exceeded that calculated from the blood iron content.

**AGRANULOCYTIC ANGINA, A Study Based on 18 Cases With 9 Necropsies, Fitz Hugh, Jr, T, and Comroe, B I** *Am J M Sc* 185 552, 1933

The significant data of 18 cases of agranulocytic angina (pernicious leukopenia) are reported with 9 necropsies. All but 4 are dead (78 per cent mortality). Of these 4 only 2 can be considered cured.

The most promising treatment, aside from blood transfusions, seems to be pentose nucleotid (Jackson) although the author's experience with this substance has not been favorable. It seems more definitely to be useless in chronic cases of the disease.

Necropsy study has shown in more than half the cases thus examined a plentiful supply in the leukopoietic centers of the progenitors of the blood leukocytes. This virtual hyperplasia is in marked contrast to the profound peripheral leukopenia characteristic of the disease, and strengthens the previous suggestion of an hypothesis of primary "maturation arrest," rather than primary "aplasia" to account for the hematologic phenomena of the disease.

Agranulocytic angina (pernicious leukopenia) seems to be a disease entity, although it is so closely simulated by certain other conditions that its nosologic status may remain debatable until a potent specific therapy is available, or some other pathognomonic feature is discovered.

**DISTILLED WATER, Triply, The Fetish of, Elser, W J, and Stillman, R G** *J A M A* 100 1326, 1933

Over a period of four years and three months, no chill has followed the introduction of physiologic solution of sodium chloride into the veins of patients at the New York Hospital. This solution was made with New York City tap water freshly distilled once, in a Barnstead still run by steam and operated during a large part of the period at the full capacity of the apparatus. This experience demonstrates with certainty that singly distilled water can be used with safety in the preparation of solutions for intravenous administration and that "triply distilled water" is truly a "fetish" in that it is a "material object regarded with awe, as having mysterious powers residing in it and from which supernatural aid is to be expected."

#### CONCLUSIONS

The use of triply distilled water in the preparation of solutions for intravenous administration is not necessary.

The use of freshly (and properly) distilled water in the preparation of solutions for intravenous administration is essential.

Solutions introduced directly into the circulation, other than physiologic solution of sodium chloride, should be administered at a rate not to exceed 5 cc per minute.

**POLYCYTHEMIA VERA, Treatment of With Solution of Potassium Arsenite, Forkner, C E, Scott, J F McN, and Wu, S C** *Arch Int Med* 51 616, 1933

Six patients with polycythemia vera have been treated by the oral administration of relatively large doses of solution of potassium arsenite.

Distinct improvement in the clinical and hematologic manifestations has occurred in each patient in from twenty to fifty days.

The induced remissions were characterized by reduction of the erythrocyte, hemoglobin and hematocrit values to normal or nearly normal, reduction of the basal metabolic rate to normal, increase in body weight, increase in strength and subsidence or disappearance of symptoms.

The remissions may be prolonged at least for several months by continuation of the medication in reduced amounts.

The method of administration of the drug and the untoward effects due to treatment with arsenic are discussed.

The administration of solution of potassium arsenite to patients suffering from polycythemia vera constitutes a safe and reliable method for the palliative treatment of this disease

**TOE RINGWORM** Effect of Chlorinated Swimming Pool Water on, Spring, D Am J M Sc 185 775, 1933

Ringworm of the toes is such a problem among students and gymnasium habitues in general that a possible fungicidal role of chlorinated water in swimming pools merits testing. With 6 women swimmers as test objects, it appeared that:

1 It is possible for detached particles of ringworm tissue to retain viable fungi in chlorinated swimming pool water for one and one half hours. The fungi, however, may be destroyed in two hours.

2 Chlorinated water in swimming pools does not effectually reach and kill fungus between the toes during an average thirty minute swimming period.

**TUBERCULOSIS, Diagnosis of Early** Value of Monocytic Lymphocytic Index, Sullivan, M, and Jones, P H Am J M Sc 185 762, 1933

$$\text{Index} = \text{ratio of monocytes to lymphocytes} = \frac{L}{M}$$

$$\text{Average normal index} = 2.9$$

It has been demonstrated that the monocyte plays an important role in tuberculosis. An increase in the number of monocytes of the circulating blood is indicative of activity. A study by the Sabin technic of the number of monocytes and relative proportion of monocytes to that of lymphocytes, that is, the monocytic lymphocytic ratio, often proves of invaluable assistance in diagnosis. In active tuberculosis the monocytes are markedly increased, and there is a reversal of the monocytic lymphocytic index. In arrested tuberculosis there is an increase in the lymphocytes, with the total number of monocytes increased but less in number than the total lymphocytes. It has also been shown that there is a storehouse for monocytes in the tuberculous subject, which does not exist in the normal nontuberculous subject. If the total number of monocytes and the monocytic lymphocytic ratio is determined before and after the administration of tuberculin subcutaneously, the blood of patients in which a definite focal reaction is obtained will show a marked increase in the number of monocytes and a shift in the monocyte lymphocyte ratio. This simultaneous provocation of focal reaction and increase in circulating monocytes is strong evidence of the presence of tuberculosis.

**BONE MARROW Biopsy,** Custer, R P Am J M Sc 185 617, 1933

Using rigid aseptic precautions, the skin and subcutaneous tissue are infiltrated at least 15 cm. to right and left of the midline with a 2 per cent solution of novocain for a distance of 3 cm. above and below the attachment of the fourth rib. After a lapse of five minutes a sagittal incision, 4 cm. in length, is made down to, but not including, periosteum. The needle is inserted beneath the periosteum and the subperiosteal space is infiltrated with novocain over the operative field, thus all sensitive parts have been rendered anesthetic. The periosteum is incised in the line of skin incision and elevated, exposing an area slightly greater than 1 cm. in diameter. Using an ordinary trephine of 1 cm. diameter, the ventral table of the sternum is cut, the marrow cavity has been entered when the sensation of a slight downward thrust is experienced (care must be exerted not to continue drilling through dorsal table into mediastinum). The trephine is tilted slightly back and forth in sagittal and transverse planes, thus breaking the fine trabeculae of the underlying cancellous bone. The trephine is withdrawn, the bone button will come away with the drill or can be removed with tissue forceps. The margin and floor of the marrow cavity are scraped with a sharp bone curette, the curettings and button are handed to the pathologist (whose presence at operation is imperative) on a sterile sponge. The cavity is packed firmly with gauze until all oozing has ceased, the

periosteum is replaced over the bone defect, but not sutured, skin and subcutaneous tissue are approximated with interrupted No 00 plain catgut (or horsehair) sutures (in female subjects a carefully laid subcuticular suture may be preferable) Sterile dressings are applied and the patient returned to bed, dressings may be discontinued after the sutures have been absorbed (usually about the sixth day) There is eventual fibrous or osseous replacement of the bone defect and the resulting scar is inconspicuous, fine, white line

**Microscopic Technique** A tiny fragment of the curettings should be teased out and stained supravitaly

The button and remaining curettings are fixed promptly in Zenker formal solution for four to six hours, washed for an equivalent length of time in running water and decalcified in a solution of equal parts of 55 per cent formic acid and 20 per cent sodium citrate (six hours is usually sufficient, although the tissue may be left overnight without destroying the staining quality of the cells) The tissue is washed in running water for about four hours, transferred through 95 per cent alcohol (twelve hours), absolute alcohol (six hours), chloroform (45 minutes), chloroform and paraffin (thirty minutes in oven), paraffin (40 to 42° C) (fifteen to twenty minutes), paraffin (53 to 56° C) (2 changes totaling thirty minutes) and finally embedded in paraffin, being careful to embed the button with the marrow surface downward The tissue is blocked and cut in the usual fashion, preferably not over 5 micra in thickness, mounted on albuminized slides and placed in the oven for at least two hours (better overnight to insure flat sections) Paraffin is removed by 2 changes of xylol, following with the slides are transferred through the following solutions 95 per cent alcohol, 95 per cent alcohol and iodine (cherry red solution) until the mercury of the fixative is removed, 95 70, 50 and 30 per cent alcohol and finally through 3 changes of distilled water to insure removal of all alcohol Slides are then placed in the stain mixture prepared as follows

#### Solution I

Eosin fur Blut (Grubler)	1 gm
Distilled water	1 liter

#### Solution II

Azure II	1 gm
Distilled water	1 liter

(Azure II as supplied by National Aniline and Chemical Company only)

Mix solutions as follows in the indicated order

1 Distilled water	80 cc
2 Solution I	20 cc
3 Solution II	10 cc

Stain from late afternoon until the following morning Differentiate, controlling differentiation under the microscope

- 1 Alcohol, 95 per cent, until blue stain ceases to come off in a cloud
- 2 Alcohol, 95 per cent, until differentiation is complete (bone trabeculae, red blood cells, eosinophil granules are the landmarks and should be red, nuclei should be sharp and brilliant blue)
- 3 Transfer rapidly through 2 changes of absolute alcohol for dehydration
- 4 Xylol (3 changes) until clear At this time check stain under high dry power, if underdifferentiated, slide can be carried back into alcohol, if overdifferentiated, it is practically useless Decolorizing in acid alcohol and restaining are possible but results do not justify the effort, better cut new sections

**LIVER FUNCTION, A New Test of The Synthesis of Hippuric Acid, Quick, A J Am J M Sc 185 630, 1933**

*The Test*—Five and nine tenths grams of sodium benzoate dissolved in 30 cc of water is administered one hour after a breakfast consisting of coffee and toast The



patient is then given  $\frac{1}{2}$  glass of water. Immediately after taking the drug the patient voids, and then collects complete hourly specimens for four hours. These are preserved with toluene, and hippuric acid determined in each specimen. In normal adults the output of benzoic acid as hippuric acid is approximately 1 gm. or more during the second and third hours, and the total for the four hours is from 3 to 3.5 gm.

For clinical purposes a simple method has been developed which will be described as Method 1.

*Method 1*—Simpler Clinical Method. Each hour specimen is measured, transferred to a small beaker and acidified with concentrated hydrochloric acid until acid to Congo red, 1 cc. of the acid is usually sufficient. The solution is vigorously stirred until the precipitation of the hippuric acid is complete, and then is allowed to stand for one hour at room temperature. The precipitate is filtered off on a small Buchner funnel or a filter plate, washed with a small quantity of cold water and allowed to air dry. The hippuric acid thus obtained is either weighed (to the second decimal place, which is sufficiently accurate) or titrated with 0.2 N sodium hydroxide, using phenolphthalein as indicator. To obtain the total hippuric acid, one must add to the amount thus obtained the calculated quantity remaining in solution, 100 cc. of urine will dissolve 0.33 gm. of hippuric acid. In case any specimen exceeds 125 cc. it should be slightly acidified with acetic acid and concentrated on the water bath to about 50 cc. before precipitating the hippuric acid. The results are best expressed in terms of benzoic acid. To convert hippuric acid to benzoic acid, one multiplies by 0.68.

*Method 2*—This determination depends on the isolation of hippuric acid from the urine by extraction with ether, and subsequent hydrolysis by means of hydrochloric acid. The glycine thus liberated is determined by the well known formal titration. 5 to 10 cc. of urine are transferred to the extraction tube of a continuous extractor, 1 cc. of 5 N sulphuric acid and 2 drops of 10 per cent sodium tungstate added. The sample is extracted with ether for ninety minutes. The ether is removed by distillation, 10 cc. of 20 per cent hydrochloric acid is added to the crystalline residue, and the flask is connected with an air condenser. The solution is refluxed on a hot plate for one hour, and then is transferred to a small dish, and evaporated to dryness on a water bath. The residue is dissolved in about 20 cc. of hot water, a small amount of decolorizing charcoal (Norit) added, and the solution filtered. The residue is washed several times with hot water. One drop of 1 per cent neutral red indicator is added to the filtrate, which is then neutralized to  $P_H$  7. At this  $P_H$  neutral red is slightly pink, i.e., midway between yellow and red. Six drops of 1 per cent phenolphthalein and 10 cc. of neutralized 40 per cent formaldehyde are added, and the solution titrated with 0.1 N sodium hydroxide until the deep red color matches the standard, which is prepared by mixing 20 cc. of distilled water, 10 cc. of neutralized formaldehyde, 6 drops of phenolphthalein, 1 drop of neutral red and 0.3 cc. of 0.1 N sodium hydroxide. Since 1 cc. of 0.1 N sodium hydroxide is equivalent to 1 cc. of 1 N glycine, the titration value, corrected for the 0.3 cc. blank, can be converted directly either to hippuric or benzoic acid.

Diminished excretion of hippuric acid was observed in luetic cirrhosis, catarrhal jaundice and in obstructive jaundice of moderately long standing. Normal results were obtained in cholecystitis and in 2 cases of portal cirrhosis.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughan, Professional Building, Richmond, Va

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### Practical Hematological Diagnosis

THIS book, both because of the comprehensive survey of its subject matter and especially because of the very practical manner in which it is covered, should prove of especial value to the practicing physician from whom it deserves, a generous reception.

So many and diverse have been the developments in the laboratory study of the blood that it is sometimes a matter of difficulty to evaluate their results and significance.

In this volume Drs. Pepper and Farley have presented in an eminently clear and utilizable manner the more salient facts concerned with the cytological examinations of the blood. Although methods for the more commonly used procedures are described in clear detail, the book is not a laboratory manual but in greater part is devoted to a discussion of the significance of blood findings and their clinical application to the study of disease.

The book is divided into three parts, the first of which describes the components of the blood in accordance with their hematological interest, selected methods for their study, and the practical significance of their determination.

Part II discusses in an orderly, detailed and satisfactory manner the diseases of the hematopoietic system and the hematological findings applicable to their diagnosis and study while Part III discusses the hematology of diseases not primarily of the blood.

A section of great interest and great value to the practitioner is that in which is described the outstanding features of the blood picture in nearly 400 diseases and conditions alphabetically arranged so that the information is readily accessible.

An extensive bibliographical index (ten pages) testifies to the thoroughness with which the literature has been covered and the reputation of the authors assures an authoritative presentation.

This volume should find a wide field of usefulness.

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### Arteriosclerosis†

THAT a man is as old as his arteries, and that arteriosclerosis is the inevitable and, as it were, normal concomitant and aftermath of the process of aging have long been more or less generally accepted axioms.

Like many another in the history of medicine these, too, have given way to the concept that arteries, perhaps, are only as old as their environment, a concept suggested by the absence of arteriosclerosis in the presence of extreme old age and its occasional postmortem demonstration in children.

It is, of course, natural that a condition so commonly encountered and responsible for such widespread secondary lesions and important functional failures should long have attracted the interest of investigators. Unfortunately, however, morphologic studies have added but little to the understanding of the problem and, indeed, may now be said to have reached an impasse. It has become increasingly evident, not only that degenerative lesions constitute

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\*Practical Hematological Diagnosis. By O. H. Perry Pepper, M.D., Professor of Clinical Medicine, Univ. of Pa. and David L. Farley, M.D., Associate in Medicine, Univ. of Pa. 562 pages, 3 colored plates. Cloth. Philadelphia: P. W. B. Saunders Co.

†Arteriosclerosis. A Survey of the Problem. Edited by Edmund A. Cowdry. Cloth pp. 617, 88 figures. New York: The Macmillan Co.

one of the most important of problems in medicine and human economy, but also, that new avenues of approach to their study must be formulated which will take into account the advances of biochemistry and the concept that arteries and the cardiovascular system in general are living things and maintain a dynamic relationship to other organs.

With these thoughts in mind, under the auspices of the Josiah Macy Jr. Foundation, a thorough survey of the problem of arteriosclerosis as a whole was undertaken, the results of which are presented in this volume edited by F. A. Cowdry of Washington University.

The purpose of the survey was threefold:

(1) To determine the data concerning arteriosclerosis which can be regarded as established and their relation to one another, (2) To discover and to definitely formulate the principal problems awaiting solution, and, (3) To suggest the best means of attacking them.

The volume properly starts with an historical resume by F. R. Long of the development of our knowledge of arteriosclerosis, followed by an account of the structure and physiology of blood vessels (F. A. Cowdry), and the physical properties of blood vessels in health and disease (C. Bromwell).

The technique of micromincration for the study of the mineral constituents of blood vessels is then described by its originator (A. Polakard).

The statistical data of arteriosclerosis is surveyed by E. Sidenstricker and the occurrence of arteriosclerosis in lower mammals and birds and its relation to the disease in man discussed by H. Fox.

Having thus outlined the broader aspects of the problem, as it were, the remaining chapters are concerned with experimental aspects and cover: Race and Climate as Possible Factors (P. Stocks), Nutrition in Relation to Arteriosclerosis (S. Weiss and G. R. Minot), Pathogenesis (W. Ophuls), Experimental Arteriosclerosis in Animals (N. Anitschkow), The Chemistry of Arteriosclerosis (H. G. Wells), Acute and Chronic Infections as Etiological Factors (W. G. MacCallum), Retinal Arteriosclerosis (J. S. Friedenwald), Arteriosclerosis of the Brain and Spinal Cord (S. Cobb and D. Blain), Coronary Arteriosclerosis (H. Karsner), Sclerosis of the Pulmonary Arteries (H. Karsner), Arteriosclerosis of the Abdominal Viscera and Extremities (E. T. Bell), Hypertension in Relation to Arteriosclerosis (F. Lange), Hereditary Aspects of Arterial Hypertension in Relation to Arteriosclerosis (G. D. Williams), The Treatment of Arteriosclerosis (J. H. Wyckoff), and a final Summary by A. E. Cohn.

An author index and subject index complete the volume.

It can be said with confidence that this volume constitutes the most complete, the most authoritative, and the most illuminating and outstanding contribution to the study of arteriosclerosis yet made. Student, investigator, pathologist, physician at large, all can read it with profit. Without doubt it will remain an outstanding reference work for some time to come.

In format and general workmanship the volume leaves nothing to be desired.

### The Elements of Medical Treatment\*

THIS book represents the subject matter of a lecture course on elementary therapeutics and has for its purpose the presentation of basic principles and their application to the commoner diseases met with in practice with special attention to the prescription of drugs.

Emminently practical in purpose, it is equally practical in the handling of the subject and may be recommended with confidence to the physician as well as the student. Those who master the contents of its twenty-four chapters will never be at loss when confronted with the usual exigencies of medical practice in general.

\*The Elements of Medical Treatment. By Robert Hutchinson M.D. Physician to the London Hospital. Cloth p. 188. Baltimore: William Wood and Co.

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## EDITORIAL

### Divinyl Oxide in the Twilight Zone

ONE of the greatest chapters in human history is that which deals with the discovery and use of anesthesia. In comparison with this, how pale and insignificant to us appear such incidents as the ascent of Moses up into the mountain to meet Jehovah face to face, the flight of Elijah up to Heaven in a chariot of fire, or the conquering of the world by Alexander the great. And it may not be too much to surmise that the medical historian of a few generations hence may also add to this list the fall of the Roman Empire, the crucifixion, the rise of the papacy and the world war. For anesthesia will spread its wings like a conquering angel over pain so long as man shall suffer.

Like most things in this life the anesthesia of today is not yet perfect. There is still ample room for improvement. And, thanks to a numerous company of eager workers, our conceptions, our ideals, and our accomplishments in anesthesia are constantly advancing toward a happier and more effective goal.

And now like the flitting fragrance of some new fruit from California come whispering echoes of a new anesthetic still lingering in the offing at San Francisco. In that city, where Dr. C. D. Leake<sup>1</sup> and his colleagues work, and in Edmonton, Canada, where Drs. Gellan and Bell<sup>2</sup> have studied this compound, it is known as divinyl ether, or divinyl oxide. But it seems probable that we may presently hear much more of this preparation under another name (vinesthene).<sup>3</sup> Vague opinions, good, bad and indifferent, regarding this compound have reached the writer. And it is obvious, that, for the moment, the drug must appear to pharmacologists as a hopeful vision in the twilight. It is a little too early yet to determine whether this is the twilight of the evening, or that which precedes the coming dawn. But it appears reasonable to believe that enough good work has now been done to indicate that this preparation promises something and perhaps a great deal, for medicine in the future.

The drug is about as explosive and inflammable as ether, and on exposure to light and air it tends to polymerize with the formation of a gel and later a resin. This latter reaction is hastened by acids but may be prevented by faint traces of alkali. Major has also observed that partial decomposition may lead to the formation of some formaldehyde and formic acid. The drug is more volatile than ether, it boils at 23.8° C. and its specific gravity is 0.77. It decolorizes potassium permanganate solutions and this reaction may lead to its detection in various biologic fluids, and even urine, in which some of the drug appears to be excreted although the greater quantity seems to be rapidly eliminated by the lungs.

Only chemically pure samples should be used for anesthesia, since those which have undergone slight decomposition and contain small amounts of formaldehyde and formic acid are irritating and dangerous. This latter feature may serve as a useful guide in the detection of deteriorated samples, and it may be added that such samples also tend to become cloudy in addition to acquiring a stinging odor. Samples improperly made or not sufficiently purified may contain chlorinated ethers as impurities, as the drug is made from chlorinated ethyl ether.

The preparation volatilizes readily, and when given by the open drop method it is absorbed by the lungs quickly and loss of consciousness and relaxation may be attained much earlier than with ethyl ether. The drug has a strong sweetish taste but is less irritating than ether and leaves no biting or burning sensation after inhalation. When pure its sweetish, ethereal odor is not so pungent and disgusting as that of ethyl ether. A new method of preparing the compound has recently been developed by Rugh and Major,<sup>4</sup> and it appears that this process yields a practically pure compound. It seems highly probable that use of slightly deteriorated samples would lead to respiratory complications. But first class samples are less irritating, less nauseating and less stormy and lasting in their action than is ethyl ether. There is less salivation induction is usually quicker and smoother, and postanesthetic nausea and vomiting are apparently less than with ethyl ether. The minimal certain anesthetic concentration for mice is 3.9 volumes per cent, while the minimal certain anesthetic concentration of ethyl ether is 4.7 volumes per cent.

With twice or more of the above respective concentrations danger appears in about the same degree with the use of each drug. It may be noted that with the minimal effective concentration of each, anesthesia appears quicker with divinyl oxide than with chloroform. Respiratory failure is the immediate cause of death in fatal cases under divinyl oxide, and in these the heart and circulation appear to be in very good condition at the time of respiratory cessation. Apparently resuscitation may be effected more easily and more certainly than after comparable degrees of poisoning with ethyl ether. If administered with deficient oxygen divinyl oxide may cause a slight rise in the hydrogen ion concentration and sugar content of the blood. With sufficient oxygen this does not occur.

Dogs anesthetized for periods of thirty minutes daily for ten days with no anoxemia showed practically no pathologic changes in the liver, heart, or other internal organs with the possible exception of some slight effect on the kidneys and urine secretion. With anoxemia the kidneys seem to be the organs more likely to undergo pathologic changes. This may be due to some excretion of the drug by the kidneys. The liver seems free from any such changes as might be produced by chloroform.

Dr Gelfan<sup>2</sup> reports anesthetization of himself twice for periods of ten and eighteen minutes respectively with divinyl oxide, while Dr Wimfred Hughes has been anesthetized with the drug for five minutes. On each of these occasions the subjects recovered and were able to converse rationally and to get up and walk about the room with full coordination in about two minutes after administration of the drug was stopped. No after-effects whatever were noted. This amount of clinical evidence is, of course, pathetically small, but it merely illustrates the extreme difficulty which one (and particularly a laboratory worker) may have in getting his discoveries tried out clinically. And this is the more pathetic when one reflects on the actual status of medical practice in general and of surgery in particular, in the world today.

The formula for divinyl oxide is  $\text{CH}_2=\text{CH}-\text{O}-\text{CH}=\text{CH}_2$ , and on the basis of its chemical similarity to both diethyl ether and ethylene Dr Leake<sup>3</sup> predicted that it should possess comparable pharmacologic properties. It seems from all the evidence at hand that the drug bids fair to fulfill this prediction. In the meantime others look on with mingled feelings of doubt and hope, and perhaps with a typically racial complex involving in the very faintest degree a few other historically human sentiments, while three or four workers shoulder the responsibility of hustling this drug off to an ignominious oblivion or of raising it to a position of respect or even of high honor in the surgical armamentarium of the immediately approaching future.

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## *CLINICAL AND EXPERIMENTAL*

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### ON COLLOIDAL CHANGES PRODUCED BY SEVERAL ANTIPYRETICS AND ANALGESICS AND THEIR ALLEGED CONNECTION WITH THE EXCITABILITY OF NERVOUS CENTERS\*

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OTTO FURTH, M D, AND RUDOLF SCHOLL, M D, VIENNA

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#### I LEADING IDEAS AND PROBLEMS

IN A RECENTLY published paper<sup>1</sup> we were able to show, that several substances exhibiting an antipyretic or analgesic power, like phenyleinchoninic acid, salicylic acid, gumine, antipyrine, phenocoll, and melubrin, increase considerably the spontaneous coagulation of tissue proteins contained in extracts of muscle, liver, and brain. It seems very probable, that some colloidal changes (especially the degree of hydration) of the proteins, which are building up certain nervous centers, may be concerned in the physiologic and pharmacologic action of those substances.

These investigations have recently increased in interest, owing to the papers of W D Bancroft,<sup>2</sup> and his associates, bearing on the reversible coagulation of living tissues. These researches deal especially with the relation of the degree of swelling of brain proteins to narcosis and the efficiency of analgesics. Sodium rhodanate, which is placed at the head of the lyotropic Hofmeister series of anions and which excels by its efficiency in peptizing proteins, is claimed to act as an antagonist toward morphine. On the contrary sodium tartrate, which stands near the opposite end of the Hofmeister series, is supposed to be a synergist of morphine. The author claims (based as well upon experiments on animals as upon observations on human beings) that in addition to morphine as well as to certain other poisons (ether, alcohol,

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\*From the Institute of Medical Chemistry of Vienna University.  
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cocaine, chloral) the colloids of the brain are in a state of increased aggregation (agglutination) and that they can be reduced to a normal state by agents, which, like sodium rhodanate, are able to produce a state of increased dispersity or hydration. Further, the author makes responsible for certain kinds of mental disturbances (such as maniac depressive insanity), an overcoagulation of the brain colloids, on the contrary he makes responsible for certain other types of insanity (such as stuporous catatonia) an overpeptization of brain colloids.

It seems quite evident that, in view of the huge complexity of nervous and psychic phenomena, simple explanations do not suffice. On the other hand it is also obvious, that the degree of swelling of the colloids of certain nervous centers must be of importance for all kinds of phenomena (irritation and stimulation) in these centers. The attempt to influence the same centers by means of drugs, therefore, seems sufficiently justified and not without good prospects. We dare say that we are standing here at the entrance to a new field of work, which seems rather promising and tempting.\*

With the view of preparing a solid foundation for future work, we first tried to ascertain for a number of antipyretics and analgesics, whether they act on colloidal systems as coagulating agents (decreasing dispersion) or as peptizing agents (increasing dispersion). It goes without saying, that only water-soluble substances can be used in this kind of investigation. As colloids suitable for our purposes, we chose proteins of the brain and muscle, gelatin, fibrinogen, lecithin and cephalin. Besides we extended our research work to India ink, as a type of a simple suspension, and to Congo red, as a type of a high molecular pigment forming a watery pseudosolution.

Among the substances causing a change in the degree of aggregation (hydration) phenylephedronic acid (cinchophen) and salicylic acid required a particular interest, inasmuch as the anions of these substances stand beyond the head of the Hofmeister anionic series and as they, provided that the reaction is neutral or alkaline, exhibit a strong peptizing power with regard to proteins. The fact that salicylates are even superior to rhodanates in their faculty of increasing the swelling of fibrin and gelatin and that they therefore stand beyond the end of the Hofmeister series, was first discovered in this laboratory by Robert Willheim.<sup>4</sup>

A further part of our investigations referred to the synergism and antagonism of antipyretics and analgesics, taking the fall of temperature caused in guinea pigs as an index. The leading idea was this: let us assume that two substances are synergists with regard to colloidal systems, influencing the state of swelling and aggregation in the same way and in the same direction. Let us further assume that the state of colloids within certain nervous centers controlling the body temperature of guinea pigs, is partly responsible for the irritability of these centers. Then it will be perfectly logical to expect, that a physiologic synergism might correspond to the colloidal synergism of anti-

\*We may quote in behalf of this view the fine results of Dr. Alexandra Adler<sup>1</sup> under the guidance of Professor Otto Pötzl recently obtained in the Psychiatric Clinic of Vienna University with Euphylline, an agent powerfully influencing the hydration of the proteins of living tissues. In twelve morphine addicts the sudden withdrawal of morphia produced only insignificant phenomena of abstinence.



pyretics, as well a physiologic antagonism might be expected to correspond to a colloidal antagonism. If Professor Bancroft's ideas are correct, one might expect such an harmony between colloidal and physiologic phenomena. If our observations fail to confirm such a simple, clear and undoubted coincidence and harmony (and they do fail, 'indeed') we shall be compelled to conclude, that these matters are far more complicated than Professor Bancroft claims them to be, and cannot possibly be forced into a simple pattern of colloidal chemistry. Nevertheless, we do not deny in the least the importance of colloidal phenomena in the irritability and stimulation of nervous centers.

## II THE INFLUENCE OF ANTIPIRETIC AND ANALGESIC AGENTS ON COLLOIDAL SYSTEMS

We first had to select a series of water soluble antipyretics and analgesics, which might be looked upon as representatives of the most important types of this kind of drug. We chose the following substances: (a) cinchophen sodium (the sodium salt of phenylquinolincarboxylic acid), (b) sodium salicylate, (c) quinine hydrochloride, (d) phenocoll (so called soluble phenacetine), hydrochloride of amino-aceto-phenetidine, as a representative of the phenacetine series, (e) melubrin (so-called soluble pyramidon, sodium salt of phenyl-dimethyl-pyrazolon-amidomethansulphonic acid, as a representative of the pyramidon series), (f) morphia, (g) novocaine, (h) cocaine, (i) chloralhydrate, (k) amylenhydrate (tertiary amyl-alcohol), (l) paraldehyde, (m) urethane.

The *colloids*, on which the coagulating or peptizing power of these agents was to be tested, were prepared in the following manner:

a *Brain proteins*—One fourth kilogram of fresh calf's brain was finely minced, mixed with  $\frac{1}{2}$  liter of physiologic saline, shaken for two hours in the shaking apparatus, strained through muslin. The resulting emulsion was saturated with powdered ammonium sulphate. The precipitate, consisting of a mixture of lecithides and proteins, was collected on a filter paper, taken from the filter, ground up with water and filtered. Thus an opalescent solution of water soluble proteins was obtained.

b *Muscle Proteins*—They were prepared in a very simple manner, by allowing minced lean beef to stand with double the volume of physiologic saline for one day. The resulting filtered, opalescent fluid contained abundant quantities of those muscle proteins, which are soluble in dilute saline solutions (myosin and myogen).

c *Fibrinogen*—Nine hundred cubic centimeters of bovine serum were allowed to run directly from the artery into a vessel, containing 100 cc of 2 per cent potassium oxalate solution in order to prevent clotting. The blood corpuscles were separated by the centrifuge. From the separated plasma fibrinogen was precipitated by the addition of an equal bulk of saturated sodium chloride solution, the precipitate was collected on a dry folded filter paper, then readily and completely redissolved in a 5 per cent sodium chloride solution. The resulting clear fibrinogen solution proved to be stable for weeks, when kept in a refrigerator.

d *Lecithin and Cephalin*—Stable emulsions of lecithin ex ovo (Merck) were prepared by dissolving 2 gm of sodium oleate in 100 cc of water and adding a hot solution of 4 gm of lecithin in 20 cc of alcohol. Thus a stable 4 per cent emulsion was obtained which was alkaline toward litmus and could be diluted at discretion. Very stable emulsions were also obtained by the procedure of O Porges and E Neubauer. 14 gm of lecithin ex ovo were dissolved in 100 cc of ether and the solution added to 100 cc of water, the mixture was

shaken for a while, then freed from ether by electrical heating and from separated lumps by filtering through glass wool. Thus emulsions were obtained, which were stable for several days and fit for dilution.

For preparing lecithin and cephalin emulsions from brain,  $\frac{1}{4}$  kg of calf's brain was ground up with 50 gm of quartz sand, dried under a pressure of 40 to 60 mm. and at  $40^{\circ}$ , then permanently shaken with 250 cc of benzol, containing 5 per cent alcohol. The separated benzol was distilled off in vacuum, there remained a waxy residue, which was suspended in ether and precipitated by alcohol. The precipitate when shaken with water yielded an emulsion of the cephalin fraction, the alcoholic ether containing filtrate, when poured into water, yielded an emulsion of the lecithin fraction of the brain.

e *Gelatin*—The increased or decreased intake of water into a gelatin jelly was studied by means of the method of Tomita.

f *India Ink*—A ten times diluted preparation of India ink ("Pelikan") was used as the paradigm of a true suspension, the separation of which was observed under the influence of added drugs.

g *Congo Red*—A 0.01 per cent watery solution of Congo red was used as a type of pseudosolution of a high molecular organic substance, which by the addition of phosphate buffer solutions could easily be standardized to a certain  $P_H$ . For instance,  $\frac{1}{2}$  cc of Congo solution +  $\frac{1}{2}$  cc m/15  $KH_2PO_4$  + 1 cc of the sample corresponding to  $P_H$  4.53 -  $\frac{1}{2}$  cc Congo solution +  $\frac{1}{2}$  cc m/15  $Na_2HPO_4$  + 1 cc of the sample corresponding to  $P_H$  8.30.

The arrangement of our numerous series of experiments was performed in the way generally used in colloidal chemistry. It seems sufficient to describe in a detailed manner only one of our numerous series of experiments, and to summarize the results of the rest.

#### ILLUSTRATIVE RECORD

In three series of experiments the precipitant or solvent action of members of the Hofmeister anionic series was compared at room temperature with several antipyretic, analgesic, and hypnotic drugs, by adding 2 cc of the sample with 1 cc of the colloidal test solution, viz (a) brain protein solution, (b) cephalin emulsion, (c) lecithin emulsion. All the samples of the tested drugs were  $\frac{1}{5}$  molecular solutions, only the hydrochlorate of morphine corresponded to a  $\frac{1}{10}$  molecular solution. There were 24 solutions to be examined. Cinchophen sodium, sodium salicylate, sodium rhodanate, potassium iodide, potassium nitrate, potassium bromide, potassium sulphate, sodium potassium tartrate, neutral sodium citrate, sodium fluoride, sodium benzoate, antipyrine, quinine hydrochloride, phenocoll, melubrin, morphine hydrochloride, amylenhydrate, chloralhydrate, paraldehyde, ethylurethane, novocaine hydrochloride, cocaine hydrochloride, medinal (sodium salt of diethylbarbituric acid), and finally, physiologic saline as a control.

a. *Brain Protein Samples*—After half an hour the cinchophen test contained a heavy precipitate. Also the phenocoll test was already precipitated. The salicylic test showed a dense turbidity, but no precipitate. All the other tests were still clear. The following day also the salicylic test was precipitated, but the precipitate was not as rich as in the cinchophen test. Also the samples containing antipyrine and melubrin (soluble pyramidon) had deposited scanty precipitates. The chloralhydrate test and cocaine test were somewhat turbid. All the other tests were clear. Two days later also the chloralhydrate and cocaine tests were precipitated and also in the novocaine test there was a precipitate.

b. *Cephalin Samples*—After half an hour the quinine and melubrin tests contained thick precipitates, the morphine and cocaine tests were turbid. The tests with cinchophen, salicylic acid, sodium rhodanate, antipyrine, amylenhydrate, chloralhydrate, paraldehyde and urethane, were on the contrary more translucent than before. The other samples remained unchanged even after two days.

*c* *Lecithin Samples*—Half an hour after the beginning of the experiment the precipitation of the morphine, melubrin and novocaine test was quite evident. The gummic, phenocoll, and sodium benzoate tests were very turbid besides these tests were turbid, the anions of which are standing at one end of the Hofmeister series, viz., sulphate, tartrate, citrate, and fluoride. A few hours later there were precipitates in these samples. A decided clearing up, still increasing in the course of the following hours, was to be noticed in the samples with cinchophen, salicylic acid, rhodanite, antipyrine, chloralhydrate, amylenhydrate, paraldehyde, urethane, cocaine.

The essential results of our observations are summarized in Table I. The sign *plus* means precipitation, the sign *minus* means clearing up of the sample, the sign *zero* means that there was no change, the sign of *interrogation* means that the results were changing or not beyond doubt.

TABLE I

	BRAIN PROTEINS	MUSCLE PROTEIN	FIBRIN OGEN	GELATIN SWELLING	LECITHIN CEPHALIN	INDIA INK	CONGO RED
Cinchophen sodium	- + +	+ + +	+	increased	~	0	0
Sodium salicylate	+ +	+ +	0	increased*	-	0	0
Potassium thiocyanate	+	+	0	increased	-	0	0
Antipyrine	+	?	0	0	-	0	0
Quinine	+	+ + +	+ + +	diminished	+ +	+ +	+ + +
Phenocoll (soluble phenacetine)	+	+	+	0	+	+ +	+ +
Melubrin (soluble pyramidone)	+	+	0	diminished	+ +	0	+ +
Morphine	0	0	0	diminished	+ +	+	+ +
Novocaine	0	0	+	diminished	+ +	+	+ + +
Cocaine	?	?	0	increased	?	0	+ + +
Chloralhydrate	+	+	0		-	0	0
Amylenhydrate, paraldehyde, urethane	0	0	0		-	0	0
Sodium sulphate, tartrate, citrate, fluoride	0	-	+	diminished	+	0	0

\* According to Popoff and Selsoff<sup>1</sup> gelatin is able to bind in presence of salicylate acid 30 per cent more water than it is the case in pure water.

The perusal of Table I reveals that the anions of phenyleinchronic acid, salicylic acid and thiocyanic acid, standing near the head of the Hofmeister series, are to be considered as synergists from the aspect of colloidal chemistry, inasmuch as they change the most different colloidal systems in the same sense, either in the sense of increased aggregation, or in the sense of diminished aggregation. Compared with these anions, not only the anions standing near the opposite end of the Hofmeister series (sulphate, tartrate, citrate, fluoride) appeared to be colloidal antagonists, but also morphine and novocaine\*. We see for instance, that cinchophen, salicylate and rhodanate produce clotting of muscle plasma with the greatest speed, whereas they are not able to precipitate India ink suspensions or Congo red pseudosolutions. They even clear up the emulsions of lecithin and increase the intake of water into a gelatin jelly. Morphine and novocaine behave on the contrary in a quite opposite manner. These substances are unable to produce clotting of the muscle plasma, but they on the contrary precipitate India ink suspensions and Congo

\* According to S. Boer<sup>10</sup> in frog's muscle sodium rhodanate causing a contraction and novocaine causing a relaxation are behaving like antagonists.

ied pseudosolutions as well as the emulsions of lecithin or cephalin. They produce dehydration of gelatin jellies. Quinine and phenocoll again partly agree with the phenylcinchoninic, salicylic and rhodanic anion from the point of view of colloidal chemistry, inasmuch as they are strongly precipitating tissue proteins. In another respect they behave as partial colloidal antagonists toward these anions. For they exhibit a very strong precipitating power toward lecithin emulsions, India ink suspensions and Congo red pseudosolutions\*. Cocaine coincides with morphine and novocaine as to its faculty of strongly precipitating Congo red solutions†.

### III THE INFLUENCE OF ANTIPYRETICS AND ANALGESICS ON THE BODY TEMPERATURE OF ANIMALS, COMPARED WITH THEIR BEHAVIOR TOWARD COLLOIDAL SYSTEMS

The starting point of the following observations was the faculty of cinchophen to cause a transitory fall of temperature in animals. This was first established by Starkenstein<sup>8</sup> and then further studied by numerous experiments performed in our laboratory.<sup>1, 9</sup> This fall of temperature is a delicate test for changes within certain nervous centers controlling the body temperature. We found ourselves face to face with the problem, how this fall of temperature is influenced by antipyretic and analgesic agents and whether there exists any interrelation between this pharmacologic interference on one side and the physicochemical interference of the same substances with the state of colloidal systems on the other side.

We used as paradigm for experiments of this kind the interesting observations on the temperature lowering effect of novocaine, which were recently performed by Ernst Pick together with Susi Glaubach<sup>11</sup> in the Pharmacological Institute of Vienna University. Novocaine causes in normal rabbits only an insignificant fall of temperature, but in guinea pigs there is a decided fall of temperature. After preadministration of thyroxin there is but a slight decrease of temperature in guinea pigs caused by novocaine. There is again the usual fall of temperature, provided that ten to twelve days have passed since the last injection of thyroxin‡.

In order to avoid prolixity only one of our numerous experiments, which all were recorded graphically, may be described in detail, whereas all the rest may be compressed into a table (Table II).

#### ILLUSTRATIVE EXAMPLE

*Synergism of cinchophen with quinine*—(Two analogous experiments on two guinea pigs.) An animal received a subcutaneous injection of 0.1 gm. per kilogram of quinine hydrochloride. No fall of temperature. Three days later it received an injection of 0.3 gm. per kilogram cinchophen sodium. There was a severe fall of temperature to 35° and the

\*We have established that no adsorption of quinine to the particles of India ink takes place.

†We wish the foregoing observations on total or partial colloidal synergism or antagonism might simply be considered as our observations, but by no means as definite dogmatic statements. Every expert in the field of colloidal chemistry knows besides sufficiently to what extent observations bearing on the precipitation of colloidal systems may be modified and even falsified by scanty nonessential and hardly controllable factors such as slight changes in the present concentration of hydrogen ions by traces of contaminations and by the presence of other ions etc.

‡In connection with these observations it seems worth mentioning that we observed on two hyperthyroidized rabbits after injection of large cinchophen doses instead of the usual fall of temperature, a decided rise of the body temperature.

temperature did not reach the normal level again before four hours. One day later the animal again received an injection of quinine hydrochloride of 0.1 gm per kilogram. Wherein formerly this dose of quinine had been without any effect, the excitability of certain nervous centers was now so far increased owing to the preadministration of cinchophen, that a sudden fall of temperature ensued even below  $34.3^{\circ}$  (the limit for reading the scale of the thermometer used for rectal measurement). It lasted five hours until the normal level of temperature was restored.

The injection of quinine was repeated the following day, i. e., two days after the injection of cinchophen. There was again a fall of temperature below  $34.3^{\circ}$ . Three and one half hours afterward the column of the thermometer had not yet risen above  $34.3^{\circ}$ , and it was not before seven hours that the normal level was reached again.

The following day the sensitivity toward quinine was tested anew. Though three days had already passed since the injection of cinchophen, the after effect was still an amazing one: the temperature fell below  $34.3^{\circ}$  and remained for seven hours on a diminished level.

Now we waited for a further four days and it was not before seven days had passed since the injection of cinchophen, that the injection of quinine was repeated. Now finally the after effect of cinchophen was over and there was no longer a fall of temperature.

In another experiment the injection of 0.1 gm of the quinine salt still produced a severe and lasting fall of temperature 1, 2, 3 and 4 days after the preadministration of cinchophen. Even after 6 days there was still a fall of temperature of  $1.8^{\circ}$  for several hours.

Other experiments revealed that, if 0.1 gm per kilogram of quinine hydrochloride was injected one day after preadministration of cinchophen

- after 0.3 gm per kilogram of cinchophen there was a fall of temperature
- after 0.2 gm per kilogram of cinchophen there was a fall of temperature
- after 0.1 gm per kilogram of cinchophen there was a fall of temperature
- after 0.05 gm per kilogram of cinchophen there was no fall of temperature

One tenth gram cinchophen per kilogram therefore was still sufficient, but 0.05 gm per kilogram was no longer sufficient for sensitizing the nervous centers and making them sensitive for a subsequent injection of quinine.

#### IV DISCUSSION OF THE RESULTS

Let us return to our starting point! Do our observations afford any support for Professor Bancroft's ideas as to the possibility of influencing nervous centers by interferences, altering the physicochemical conditions of their colloids? (We dealt with the centers, controlling the body temperature of guinea pigs and rabbits.)

We select from our 21 experiments those seven which afford clear and undoubted evidence of *physiologic synergism*. Was there always and regularly also the evidence of a *colloidal synergism*? This is in fact by no means the case.

Cinchophen	+ quinine—partial colloidal synergism
Cinchophen	+ morphine—total colloidal antagonism
Salicylic acid	+ quinine—partial colloidal synergism
Salicylic acid	+ morphine—total colloidal antagonism
Salicylic acid	+ novocaine—total colloidal antagonism
Quinine	+ morphine—partial colloidal synergism
Quinine	+ novocaine—partial colloidal synergism

It is instructive to note the prevention of the fall of temperature caused by cinchophen by preadministration of salicylic acid. This is a case of evi-

TABLE II

NO	COMBINED PHARMACOLOGICALLY ACTIVE SUBSTANCES	THEIR INTERRELATION WITH REGARD TO COLLOIDAL CHEMISTRY colloidal synergism or antagonism?	ANIMALS USED IN THE EXPERIMENT	TEMPERATURE IN EXPERIMENTS	RESULT OF THE EXPERIMENTS physiologic synergism or antagonism?
1	Cinchophen Na + quinine	Synergism with regard to the precipitation of proteins, otherwise antagonism	3 guinea pigs	The injection of quinine hydrochloride 0.1 gm per kilogram does not cause any fall of temperature, but there is a fall of temperature, provided that cinchophen Na 0.4 gm per kilogram had been injected two days ago. Then a subsequent injection of quinine, administered at a time when the fall of temperature caused by cinchophen was already over for a long time, induced a severe fall of temperature.	Synergism
2	Cinchophen Na + quinine	Synergism with regard to the precipitation of proteins, otherwise antagonism	3 guinea pigs	The marked sensitizing action produced by 0.4 gm per kilogram of cinchophen, lasted three days at least, but it has vanished after 7 days.	Synergism
3	Cinchophen Na + quinine	Synergism with regard to the precipitation of proteins, otherwise antagonism	2 rabbits	After preadministration of 0.5 gm per kilogram cinchophen (producing only a slight fall of temperature) 0.1 gm per kilogram quinine hydrochloride one day later produced a severe fall of temperature. The following day injection of 0.2 gm per kilogram quinine hydrochloride. There was a lethal fall of temperature.	Synergism
4	Cinchophen Na + quinine	Synergism with regard to the precipitation of proteins, otherwise antagonism	1 guinea pig	Already 0.2 gm per kilogram of cinchophen sensitizes the nervous system for 0.1 gm per kilogram quinine hydrochloride (which of itself is not able to produce any fall of temperature) and this sensitizing effect lasts 4 days.	Synergism
5	Cinchophen Na + quinine	Synergism with regard to the precipitation of proteins, otherwise antagonism	1 guinea pig	0.1 gm per kilogram cinchophen proved to be sensitizing, 0.05 gm per kilogram cinchophen failed to sensitize.	Synergism

TABLE II—CONT'D

NO	COMBINED PHARMACOLOGICALLY ACTIVE SUBSTANCES	THEIR INTERRELATION WITH REGARD TO COLLOIDAL CHEMISTRY <i>colloidal synergism or antagonism?</i>	ANIMALS USED IN THE EXPERIMENT	TEMPERATURE EXPERIMENTS	RESULT OF THE EXPERIMENTS <i>physiologic synergism or antagonism?</i>
6	Canthophen Na + quinine salicylate	Synergism with regard to the precipitation of proteins otherwise antagonism	1 rabbit	0.5 gm per kilogram canthophen (as sodium salt) induced a fall of temperature to 31°, lasting 12 hours. One day later 0.1 gm per kilogram quinine hydrochloride induced a fall of temperature to 36.1°, two days later to 35.8°. A further injection of quinine, when administered four days later remained without any effect.	Synergism
7	Canthophen Na + sodium salicylate	Total synergism	4 guinea pigs	Canthophen 0.3 gm per kilogram (corresponding to the half lethal dose) causes a deep fall of temperature below 31°, lasting for 6 to 6 hours altogether. This fall of temperature was perfectly prevented, if 0.2 gm per kilogram sodium salicylate were injected 4 hours before.	Antagonism
8	Canthophen Na + sodium salicylate	Total synergism	1 rabbit	0.4 gm per kilogram sodium salicylate, then four hours later 0.5 gm per kilogram canthophen. The expected fall of temperature was prevented at first and the temperature remained unaltered for four hours. It was not until this time, that the fall of temperature was setting in, with a delay of four hours.	Antagonism
9	Canthophen Na + sodium salicylate	Total synergism	1 rabbit	A rabbit of 3½ kilograms body weight received simultaneously 0.5 gm per kilogram canthophen (½ lethal dose) + 0.1 gm per kilogram sodium salicylate (¼ lethal dose). The fall of temperature produced by canthophen was greatly attenuated, nevertheless the animal died.	Antagonism

TABLE II—Continued

NO	COMBINED PHARMACOLOGICALLY ACTIVE SUBSTANCES	THEIR INTERRELATION WITH CHEMISTRY colloidal synergism or antagonism?	ANIMALS USED IN THE EXPERIMENT	TEMPERATURE FAHRENHEITS	RESULT OF THE EXPERIMENT, physiologic synergism or antagonism?
10	Cinchophen Na + novocaine	Total antagonism	2 guinea pigs	When the fall of temperature, produced by 0.3 gm per kilogram cinchophen was over, 0.1 gm per kilogram novocaine did not produce any greater effect on body temperature than usual	Ø
11	Cinchophen Na + morphine	Total antagonism	1 guinea pig	Morphine hydrochloride, 0.1 gm per kilogram slight rise of temperature Several days later cinchophen 0.3 gm per kilogram fall of temperature to 35.5°, lasting 1 hour Afterward the injection of morphine was repeated no effect on temperature	Ø
12	Cinchophen Na + morphine	Total antagonism	1 guinea pig	The former experiment was repeated on another animal Now the second injection of morphine caused a fall of temperature below 34.3°, lasting for 7 hours	Synergism
13	Cinchophen* + neobornaval (a compound, containing the ester of borneol + isovalerianic acid + glycolic acid)	? ? The biochemical behavior could not be established, as neobornaval is insoluble in water	5 guinea pigs	The fall of temperature caused by 0.3 gm per kilogram cinchophen was perfectly abolished by preadministration of 0.75 gm neobornaval This was also true for the fall of temperature caused by quinine after preadministration of cinchophen	Antagonism
14	Salicylic acid + quinine	Synergism relative to brain and muscle proteins Otherwise antagonism	2 guinea pigs	Neither 0.1 gm per kilogram quinine hydrochloride, nor 0.4 gm per kilogram sodium salicylate of itself produces a fall of temperature But there causes a deep fall of temperature, if, after preadministration of salicylic acid, 1 hour later quinine is injected The temperature falls to 34° and remains for many hours on the same low level	Synergism

\*This experiment was suggested by a paper by P. Wegert in Riga according to which a valentan-injection increases considerably the temperature-reducing effect of antipyrine



TABLE II—CONT'D

NO	COMBINED PHARMACO DYNAMICALLY ACTIVE SUBSTANCES	TIGHT INTERRELATION WITH REGARD TO COLLOIDAL CHEMISTRY: <i>colloidal synec- gism or antagonism?</i>	ANIMALS USED IN THE EXPERI- MENT	TEMPERATURE EXPERIMENTS	RESULT OF THE EXPERIMENTS physiologic synec- gism or antagonism?
15	Salicylic acid + morphine	Total antagonism	3 guinea pigs	Neither 0.1 gm per kilogram morphine nor 0.1 gm per kilogram sodium salicylate caused a marked fall of temperature. But there was a deep fall of temperature, if 1 hour after administration of the salicylate, morphine was injected. The temperature remained for 8 hours near 35°	Synecgism
16	Salicylic acid + novocaine	Total antagonism	2 guinea pigs	The fall of temperature caused by 0.2 gm per kilogram of novocaine was greatly increased by preadministration of 0.2 gm per kilo-gram, or 0.1 gm per kilogram of sodium salicylate two hours previously	Synecgism
17	Sodium rhodanate + morphine	Total antagonism	1 guinea pig	0.2 gm per kilogram sodium rhodanate + two hours later 0.1 gm per kilogram morphine hydrochloride: no fall of temperature	0
18	Sodium rhodanate + novocaine	Total antagonism	1 guinea pig	0.2 gm per kilogram sodium rhodanate + two hours later 0.1 gm per kilogram novocaine: no fall of temperature	0
19	Sodium rhodanate + quinine	Synecgism relative to brain and muscle proteins—otherwise antagonism	1 guinea pig	0.2 gm per kilogram sodium rhodanate + two hours later 0.1 gm per kilogram quinine: no fall of temperature	0
20	Quinine + morphine	Partial synecgism	2 guinea pigs	Neither 0.1 gm per kilogram morphine hydrochloride, nor 0.1 gm per kilogram quinine hydrochloride produces a fall of temperature by itself. But if morphine is given and then 1 hour later quinine, a fall of temperature to 34.5° ensues	Synecgism
21	Quinine + novocaine	Partial synecgism	2 guinea pigs	The fall of temperature, caused by 0.1 gm per kilogram novocaine is considerably increased, if two hours previously 0.1 mg per kilogram quinine, i.e., a dose, which does not cause any fall of temperature by itself, is given. The temperature falls, with convulsions, below 34° and the normal level is not attained before 5 to 7 hours	Synecgism

dent physiologic antagonism in spite of the fact that cinchophen and salicylic acid may from the physicochemical point of view be looked upon as perfect synergists as regards their action on colloids

It seems very probable and can scarcely be denied from the theoretic point of view, that it may not be impossible to influence the processes within the colloids of the nervous centers by physicochemical interferences. But we find in our experiments no support for the practical availability of Professor Bancroft's conceptions. Besides we must state objectively, that, so far at least as we know, they have been up to now rejected by neurologists.\*

We further tried to get more evidence on the question whether such physiologic synergisms as exist between cinchophen and quinine or between salicylic acid and novocaine, or such physiologic antagonisms as exist between cinchophen and salicylic acid, are primarily due to simple physicochemical interactions on the colloids of nervous centers or whether any more complicated processes and conditions are responsible for them. Provided that the first view is correct, we are entitled to expect that similar physiologic synergisms and antagonisms as became evident in our experiments on body temperature, might also manifest themselves in quite different fields of work quite independently from those processes which take place in nervous centers. We therefore performed several arbitrary experiments with combinations of drugs choosing three quite heterogeneous tests: (a) The reduction of the fermentative power of yeast, (b) the inhibition of an enzymatic action, viz., the peroxidase of pus, and (c) the determination of the minimal lethal dose on white mice. In none of these series of experiments was there even the slightest trace of analogous synergisms or antagonisms to be noticed, which in all probability ought to manifest themselves, provided that they were caused by physicochemical reactions, particularly colloidal interferences.

It seems superfluous to describe all these experiments in detail, the more so as the outcome was a perfectly negative one.

It seemed, no doubt, at first very tempting, to confine complicated neuro-physiologic problems according to Professor Bancroft's ideas into a series of test tubes, as used in colloid chemistry and to contemplate them as reflected by the mirror of simple physicochemical conceptions. We believe that at least for the present we must renounce this kind of expectation, as there does not seem to exist as yet sufficient underground for them. Students of nature ought to take care, not to be lured into blind alleys by the desire for simplifying their conceptions.

Otherwise we believe that the physicochemical study of the behavior of the colloids making up the nervous tissues toward pharmacologic and toxicologic

\*Sutton Moore and Hauenstein<sup>11</sup> (special narcotic committee of the Tompkins County Medical Society of New York. Sodium thiocyanate (Rhodanate) in narcotic addiction. There has been disseminated largely through the lay press considerable publicity concerning the use of sodium rhodanate as a cure for drug addiction. It is of interest to state at this time that the Medical Society has knowledge of more than a hundred cases of drug addiction treated with sodium rhodanate without success. So far as can be determined at present, there is no magic efficacy nor convincing beneficial effect of sodium rhodanate in disturbances of nervous system colloids. Sodium thiocyanate (Rhodanate).<sup>12</sup> "The work of Wilder Bancroft and his associates" has not prompted any medical investigator to confirmatory trial and according to Science<sup>13</sup> preliminary investigation in U. S. Public Health Service has failed to confirm the claims of Bancroft and his associates.

logic agents deserves some interest. The experiments described in this paper may be considered as a beginning in this line of work\*. Besides we feel that the study of synergisms and antagonisms which we have begun, using the fall of temperature of guinea pigs and rabbits as a simple test, requires a continuation and amplification. The synergism of cinchophen with quinine and morphine, of salicylic acid with both these alkaloids and with novocaine,† of quinine with morphine and novocaine, as well as the physiologic antagonism between cinchophen on the one side and neobornival and salicylic acid on the other means a certain enlargement of our knowledge. The possibility of making a practical use of these facts in the line of clinical or pharmacologic work can scarcely be denied offhand.

#### V. SUMMARY

1 Our foregoing researches, according to which certain antipyretics and analgesics, like cinchophen, salicylic acid, quinine, antipyrine are able to accelerate spontaneous coagulation of tissue proteins, especially brain protein, made it probable that colloidal changes within certain nervous centers may be concerned in the action of those drugs. The conceptions of Professor W. D. Bancroft and his associates are tending in the same direction, by explaining certain mental disturbances (particularly addiction to morphine and other drugs) as hypercoagulation of brain colloids and by trying to cure them by peptizing agents (such as sodium rhodanate).

2 The behavior of watery solutions of certain antipyretics and analgesics (narcotics) such as cinchophen, sodium salicylate, quinine, phenocoll (soluble phenacetin), melubrin (soluble pyramidon), morphine, novocaine, cocaine toward colloidal systems (brain protein, muscle protein, fibrinogen, gelatin, lecithin, cephalin, India ink, and Congo red) was studied. They behaved when compared with each other, partly as physicochemical *synergists*, partly as *antagonists*.

3 We further studied as to whether there was any correspondence between physiologic synergisms and antagonisms and physicochemical synergisms and antagonisms. The fall of the body temperature of guinea pigs and rabbits was used as a test. It became evident beyond doubt, that there is not any such parallelism. We came to the conclusion that there does not seem to be sufficient foundation for the practical availability of Professor Bancroft's conceptions, though the importance of physicochemical factors in general in the colloidal processes going on within nervous centers can scarcely be denied offhand.

4 By preadministration of 0.3 to 0.1 gm. per kilogram of cinchophen, the nervous centers of guinea pigs are sensitized for several days (when the fall of temperature caused by this drug has long since passed). This sensitization makes a dose of 0.1 gm. per kilogram quinine (which of itself is not able to reduce the temperature of normal guinea pigs) produce a pronounced fall of

\*Lately, Mona Spiegel\* studied the action of bromide on lecithin from the point of view of colloidal chemistry.

†W. Zipf† mentions in his paper bearing on muscular poisons causing contraction the antagonistic action of novocaine in regard to sodium salicylate.

temperature On the contrary the fall of temperature caused in normal animals by sufficient dose of cinchophen can be entirely prevented by preadministration of sodium salicylate or neobornival (isovaleric-glycolic-ester of borneol)

5 By combining the administration of salicylic acid with quinine or morphine temperature falls ensued, whereas the components of themselves were not able to reduce the temperature under the same conditions

6 The fall of temperature caused by novocaine in guinea pigs (*Sust Glaubach and Ernst Pick*) was markedly increased by preadministration of salicylic acid and quinine

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# A COMPARISON OF SUGAR TOLERANCE CURVES OBTAINED ON VENOUS AND CAPILLARY BLOOD\*

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IT HAS been known for a number of years that the concentration of glucose in arterial blood may be greater than that in venous blood and that its concentration in blood collected from the finger tip approaches the value found for arterial blood

Foster<sup>1</sup> showed that arterial and capillary blood samples from dogs contain approximate quantities of glucose. He also studied venous and finger tip glucose tolerance curves on human subjects and found that higher values were given by the finger-tip blood, the maximum difference being 81 mg of glucose per 100 cc of blood. This large variation was due probably to two causes: first, five minutes elapsed between the taking of the respective samples, and second, the finger-tip blood sugar method used was stated to be less accurate than the Folin and Wu method which was used for the venous blood sugar.

Rabinowitch<sup>2</sup> also found large differences between arterial and venous blood sugar values, the maximum difference was 80 mg per 100 cc of blood with an average difference of 53 mg. His subjects had shown sugar in their urine during examination for life insurance but had no clinical signs suggestive of diabetes and gave normal blood sugar time curves following the ingestion of glucose. It is questionable whether these should be considered as normal persons in this type of experiment. For diabetic subjects he found that the difference between arterial and venous samples was small, especially if the case was severe.

Friedenson, Rosenbaum, Thalheimer, and Peters<sup>3</sup> used a micromodification of Benedict's 1925 sugar method, they found the range of cutaneous-venous difference 0.5 hour after the ingestion of 50 gm of glucose to be from 8 to 50 mg (average 21 mg) per 100 cc of blood. At 1 hour the average difference was only 13 mg. Patients who had received insulin showed a slightly higher cutaneous blood sugar.

Timble and Maddock<sup>4</sup> used the new Folin ferricyanide method and found the fasting blood sugar to be the same for finger-tip and venous blood but 1 hour after meals the finger-tip blood sugar was from 5 to 27 mg higher. One person, who had exercised several hours after receiving food, showed no difference for cutaneous-venous blood sugar.

Due to the number of inquiries received from clinicians regarding the subject we felt that further study would be of value.

## THE METHOD

Fifty grams of glucose in 250 cc of water was given to students and diabetic patients after a twelve to fourteen-hour fast. Blood samples were taken

\*From the laboratory of Physiological Chemistry, University of Minnesota.  
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while the subjects were fasting and at one-half hour, one hour, two and three hours after the glucose ingestion. The microsugar method of R. B. Gibson<sup>5</sup> was used because, in our experience, it seems to give the most reliable results of any of the microsugar methods. The blood samples were taken as follows: 1 c.c. of blood was drawn from an arm vein into a syringe, the plunger was removed and 0.2 c.c. of the blood was drawn into a pipette. This was discharged into a phosphotungstic acid solution and the pipette rinsed with the solution. The finger-tip sample was obtained within one minute of the venous sample by pricking the finger with a sharp Bard-Parker, size 11, surgical knife blade. After a slight milking of the finger, the 0.2 c.c. pipette was filled, and the blood transferred to the phosphotungstic acid solution. After all samples were obtained, the analyses were completed in the usual manner. Duplicate analyses were made on 51 venous blood samples with an average variation of 1.4 mg. of glucose per 100 c.c. of blood and a maximum variation of 5 mg., therefore duplicate analyses were not made on the later samples and only one set of data is reported.

#### DISCUSSION OF RESULTS

The data given in Tables I and II were obtained from glucose tolerance determinations on 21 normal and 8 diabetic subjects. In Table I, for normal persons, it will be noted that the fasting levels as given for cutaneous and venous blood are very near the same values, except in the case of W<sub>1</sub>, in which the cutaneous result is questionable and should be discarded. With the exception of this case the maximum determined variation in fasting blood sugars is 6 mg. per 100 c.c. of blood, which is practically the same variation as was found in duplicate samples.

Different results were given by the samples obtained at half hour and one hour after ingestion of the glucose and during the hyperglycemic period. The cutaneous blood sugar was higher in all instances except three. The maximum difference was 42 mg. per 100 c.c. of blood and the difference between the averages of all cutaneous and all venous values was 14 mg. and 15.4 mg. per 100 c.c. of blood, respectively, for the two periods. The difference between the blood sugar contents of venous and arterial bloods is supposed to be due to the rapid removal of glucose from the blood by the tissues during hyperglycemia.

The samples taken two hours after the ingestion of glucose show more or less irregularity. There is some difference between the venous and cutaneous blood sugars, and some persons show a delayed glucose tolerance, that is, the blood sugar has not returned to the fasting level within two hours after the ingestion of the glucose. It appears that the venous blood sugar returns to the fasting level first, while the value for the cutaneous blood is still high. It seems that a delayed glucose tolerance is somewhat more pronounced by the cutaneous method. A three-hour sample should be taken whenever possible, as the cutaneous and venous blood sugars in normal persons are the same and a delayed tolerance will have returned to the fasting level, thus distinguishing a normal person from one with mild diabetes.

The data given in Table I indicate that the same type of glucose tolerance curve is given by cutaneous and venous bloods, especially if the three-hour

sample is taken. There may be variations at half hour, one hour, and two hours, during which time the cutaneous blood usually shows somewhat higher values, but the difference is never great enough to change the type of curve, thus one would always arrive at the same conclusion regardless of the method used to obtain the blood.

Table II gives the data obtained from mild and moderate diabetic patients in hospital wards. All of the patients were under management and no insulin

TABLE I

CUTANEOUS AND VENOUS BLOOD SUGAR VALUES DURING A SUGAR TOLERANCE TEST ON NORMAL PERSONS

(Figures expressed as mg per 100 cc of blood)

SUBJECT	FAST ING LEVEL	DIFFER ENCE	0 5 HR.	DIFFER ENCE	1 HR.	DIFFER ENCE	2 HR.	DIFFER ENCE	3 HR.	DIFFER ENCE
1 Fl	c* 110		200		138		87			
	v 107	3	182	18	107	31	90	-3		
2 W1	c 126		152		150		92			
	v 104	22	142	10	138	12	92	0		
3 Win.	c 95		165		152		91			
	v 95	0	152	13	142	10	81	10		
4 Go	c 105		155		147		122			
	v 104	1	150	5	118	29	105	17		
5 Ro	c 104		186		142		100			
	v 100	4	176	10	150	-8	84	16		
6 Eh	c 92		150		100		100			
	v 90	2	138	12	94	6	65	35		
7 Wa	c 82		173		109		77			
	v 78	4	152	21	86	23	73	4		
8 Ma.	c 78		122		135		105		68	
	v 80	-2	114	8	131	4	89	16	68	0
9 Gr	c 80		135		135		126		84	
	v 80	0	131	4	129	6	120	6	77	7
10 McE	c 94		170		200		79		96	
	v 89	5	165	5	173	27	56	23	92	4
11 W1	c 90		138		155		122		87	
	v 90	0	109	29	118	37	86	36	82	5
12 Ya	c 90		170		94		77		73	
	v 87	3	138	32	78	16	73	4	72	1
13 Gl.	c 81		135		124		77		86	
	v 80	1	122	13	110	14	68	9	86	0
14 Ga.	c 91		192		159		81		91	
	v 89	2	165	27	131	28	67	14	90	1
15 McEl	c 118		204		186		126		91	
	v 112	6	186	18	161	25	106	20	-	-
16 M1	c 79		173		142		77		69	
	v 75	4	131	42	124	18	64	13	-	-
17 Ju	c 109		135		105		100		114	
	v 104	5	121	14	97	8	85	15	-	-
18 He†	c 89		173		122		110		39	
	v 89	0	155	18	114	8	104	6	42	-3
19 Av†	c 74		186		140		118		56	
	v 78	-4	192	-6	126	14	114	4	54	2
20 Ho	c 99		176		118		102		105	
	v 96	3	182	-6	105	13	95	7	107	2
21 Go	c 110		189		133		82		94	
	v 110	0	182	7	129	4	77	5	92	2
Average	c 95		165		137 ±		97 7		79 9	
	v 92 2	2 8	151	14	122	15 4	85 4	12 3	78 4	1 5
Maximum Variation		22		42		37		36		7

\*c = cutaneous v = venous.

†Received 100 gm glucose

TABLE II

CUTANEOUS AND VENOUS BLOOD SUGAR VALUES DURING A SUGAR TOLERANCE TEST ON DIABETIC PATIENTS

(figures expressed as mg per 100 cc of blood)

SUBJECT	FAST ING LEVEL	DIFFER ENCE	0 5 HR	DIFFER ENCE	1 HR	DIFFER ENCE	2 HR	DIFFER ENCE	3 HR	DIFFER ENCE
1 Hi	c* 145		231		297		253		217	28
	v 145	0	208	23	266	31	226	27	189	
2 Oh	c 241		316		366		410		400	15
	v 236	5	289	27	359	7	400	10	385	
3 Ru	c 129		176		221		236		167	20
	v 126	3	170	6	196	25	217	19	147	
4 Bo	c 186		247		306		349		316	0
	v 204	-18	259	-12	325	-19	349	0	316	
5 Ha	c 173		241		281		337		297	8
	v 170	3	253	-12	266	15	325	12	289	
6 We	c 145		217		289		337		297	8
	v 147	2	208	9	266	23	316	21	289	
7 Jo	c 157		204		217		281		253	6
	v 157	0	196	8	200	17	253	28	247	
8 Ro	c 157		221		325		337		-	-
	v 155	2	208	13	297	28	325	12	-	
Average	c 166		232		288		317		278	12
	v 167	1	224	8	272	16	301	16	266	
Maximum variation		-18		27		31		28		28

\*c = cutaneous v = venous

or food had been given for fourteen hours before the test. The data obtained on Bo are difficult to explain as the cutaneous blood sugars during fasting and at half hour and one hour after the glucose was ingested were lower than for the venous blood sugars, but it seems that some diabetic patients respond in this manner as similar results have been reported.

The sugar in the cutaneous blood of the other diabetic patients is greater than in the venous blood throughout the prolonged hyperglycemic period. The difference in cutaneous and venous blood sugars for the diabetic patients is about the same as was found in normal persons. It is usually considered that arterial-venous blood sugar difference in diabetic patients is less than for non-diabetics, and Rabinowitch has suggested the use of this difference as an index of the degree of diabetes, a total diabetic demonstrating no difference. Probably our group of diabetic patients gave arterial-venous blood sugar differences very close to those obtained in normal persons due to their being well managed and having received insulin the day before the test.

Any effect that might be produced on the blood sugar value by excessive squeezing of the finger while taking the blood sample was investigated. Several glucose tolerance tests were run in the usual manner except that one hand was placed in cold water thus causing the finger to require much squeezing to obtain the sample while the blood taken at the same time from the other hand flowed freely. By this procedure the results obtained varied slightly, but no significant difference was found. After one becomes accustomed to taking finger-tip blood samples, with the proper technique and a sharp stylus,\* only a very slight milking

\*If the stylus used is sharp little pain is felt by the patient and the blood is less liable to clot while filling the pipette.



of the finger is required, except in rare instances. The microsugar method of R. B. Gibson is excellent for use with diabetic patients, as it gives as accurate results as can be obtained with the macromethods, less time is required to collect the blood samples, and glycolysis cannot occur as the blood is immediately placed in the phosphotungstic acid solution. All the diabetic patients and most of the normal persons preferred the use of a finger tip method.

#### SUMMARY

It has been shown that the fasting blood sugar values obtained on finger-tip and venous blood are identical.

During the hyperglycemia which follows the administration of glucose the blood sugar values given by finger-tip blood are slightly higher than those for venous blood in both normal and diabetic subjects.

In normal subjects the hyperglycemia had completely disappeared three hours after the ingestion of glucose and the values obtained in cutaneous and venous blood samples were again identical.

Squeezing the finger while obtaining the sample causes no significant variation in the result.

The type of curve and the interpretation of the glucose tolerance test are the same whether venous or finger-tip blood is used.

We wish to thank Dr. George Fahr for assistance in obtaining diabetic subjects.

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## CONCERNING THE MECHANISM OF FOCAL INFECTION\*

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IT IS now the consensus of opinion that various streptococci, staphylococci and other organisms producing toxins and other pathogenic substances in chronic localized primary foci of infection may be responsible for the production of secondary lesions elsewhere in the body. This is the fundamental basis for the subject of "focal infection" in relation to systemic disease, upon which a very voluminous literature has accumulated since the pioneer and meritorious investigations of Billings<sup>1</sup> and Rosenow.

Usually the subject of "focal infection" also conveys the idea that organisms and especially streptococci, growing in such primary foci as infected teeth, tonsils, nasal accessory sinuses, etc., may acquire a specific affinity or elective localization for certain tissues or organs as the iris, joints, nerves, muscles, etc. This hypothesis is mainly based upon the observations of Rosenow, Haden,<sup>3</sup> and others that the intravenous injection of rabbits with freshly isolated organisms from primary foci produced lesions in these animals corresponding to the location of secondary lesions in those patients from whom the cultures were obtained.

Upon this question of acquired selective affinity of streptococci and other organisms in primary foci for certain tissues a large and controversial literature has likewise accumulated since some investigators state that the intravenous injection of freshly isolated cultures into rabbits did not produce lesions corresponding in distribution to the secondary lesions of patients from whom the organisms were obtained.

However, this does not reduce the fundamental importance of focal infection in relation to systemic disease as it may be that factors other than acquired selective or specific affinity determine the secondary localizations. But the question of whether or not toxins alone produced in primary foci are capable of producing the secondary lesions rather than the organisms themselves is more pertinent and one that has curiously enough escaped the attention of investigators in this field so that little or nothing of direct value is to be found in the literature upon this subject.

Upon the suggestion of Professor Kolmer, I have therefore devoted most of the present study to this phase of the problem and have injected rabbits intravenously not only with freshly isolated cultures from primary foci but likewise with Berkefeld filtrates of these to ascertain if the latter (toxins?)

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are capable of producing lesions in rabbits comparable in degree and distribution to those produced by whole organisms

This question is not only of academic interest in relation to the mechanism of focal infection but likewise of some practical importance because if toxins are capable of producing secondary lesions the removal of their source in primary foci would be expected to yield better therapeutic results than expected if the secondary lesions were due to the actual presence of infection by organisms themselves which, if firmly established, would render them independent of the primary focus or foci

#### PURPOSE OF INVESTIGATION

The purpose of this investigation therefore was twofold as follows

- 1 To ascertain if the intravenous injection of rabbits with freshly isolated cultures of organisms from primary foci of infection produced lesions corresponding in distribution to the secondary lesions presented by patients from whom the cultures were obtained as contributing to the debatable problem of acquired selective or specific affinity of organisms in primary foci
- 2 To ascertain if the intravenous injection of rabbits with filtrates of the cultures of these organisms were capable of producing lesions and if these showed any evidence of selective localization By using sterile filtrates carrying soluble toxins it was thought possible to avoid the occurrence of embolism which some investigators claim may determine the localizations when whole cultures are injected

#### METHOD OF STUDY

The materials used in conducting the investigation were collected from 21 patients in the wards of the Graduate Hospital of the University of Pennsylvania presenting foci of infection (see Table I) These were carefully studied from all angles by the different departments of the hospital

The organisms employed in the experimental work were streptococci isolated from the infected tonsils, teeth, sinuses, and the eye Others were from cases of rheumatic endocarditis where the organisms were isolated directly from the blood The strains of the streptococci recovered and employed were as follows nonhemolytic streptococci in 12 cases, *Streptococcus viridans* in 8 cases, *Streptococcus hemolyticus* in 1 case *Staphylococcus aureus* was employed from two cases of otitis media and maxillary sinusitis

The procedures recommended by Rosenow were carried out Infected tonsils were aseptically removed from the patients and placed at once into sterile Petri dishes or sterile gauze to prevent contamination In the laboratory they were seared with a hot blade and a puncture made through the seared surface with a sterile scalpel Cultures were taken by means of a sterile swab from the purulent material deep in the crypts avoiding contamination

In infected sinuses, sterile swabs were employed for the collection of purulent exudates

The teeth were aseptically extracted and the roots clipped off with the longoum forceps and dropped at once into culture media

All cultures were made in Rosenow's glucose brain broth media under partial oxygen tension for the cultivation of streptococci. The medium was prepared by dissolving 8 gm of dehydrated bacto broth in 1000 cc of distilled water, adding 8 gm of sodium chloride, 2 gm of chemically pure glucose and after cooling, 10 cc Andrade's indicator and titrated to  $P_H$  7.4. The medium was placed in tubes 20 by 1.5 cm with three pieces of calf brain or beef brain about 1 cc and two or three pieces of calcium carbonate in the form of crushed marble to insure correct reaction. The column in each tube after the brain and marble are added was about 12 cm in height. The medium was sterilized in an autoclave at a pressure of 17 pounds (7.5 kg).

The materials from the infected tonsils, sinuses, teeth, and eye were inoculated directly into the glucose brain broth and kept at 35° C to 37° C in the incubator from eighteen to twenty-four hours when they showed growths at various gradations of oxygen tension. Preliminary smears were taken for study and the streptococci varied from short to long chains. Inoculations were made on blood agar plates for the determination of the types of streptococci present.

In preparing the *filtrates* pure cultures were inoculated in glucose brain medium and permitted to grow for six days in the incubator at 35° C to 37° C. On the sixth day these cultures were carefully filtered through sterile Berkefeld N filters with the aid of suction.

The filtrates, without the addition of preservatives, were left in the incubator for another twenty-four hours at 35° to 37° C to insure the absence of contamination during the process of filtration. At the end of the twenty-four hours, they were crystal clear when not contaminated. When contaminated, they were discarded and a new batch prepared.

The filtrates were injected intravenously into healthy rabbits in doses varying from 10 cc to 15 cc. In some animals one injection was required to produce the desired lesions and in others two injections were necessary. The second injection was given on the fourth day after the first. The animals were carefully observed each day for signs and symptoms of infection. Autopsies were performed at once upon any succumbing to the inoculations. Those that survived the injections were chloroformed and autopsied on the seventh and ninth days. A careful search was made for the gross lesions found in the organs and viscera under good and clear light. Any organ or viscera found showing pathologic lesions were removed and placed at once in 10 per cent formalin solution and Zenker's fluid for fixation. Sections were prepared by the paraffin embedding method and studied microscopically.

Another series of healthy rabbits were injected with the living organisms taken from the preliminary cultures grown from eighteen hours to twenty-four hours. The injections were given in the same way as the series with the filtrates except that the doses ranged from 8 cc to 10 cc. The cultures were thoroughly inverted three times before the injections in order to secure a uniform suspension of the organisms. In most animals one injection was sufficient to produce lesions but in others two injections were given. The second was made four days after the first. The same identical method of

observation of the animals, procedure in the performance of autopsies and preparation of tissues for microscopic studies were carried out in this series of animals as in the other where the filtrates were given

#### RESULTS

*Gross Lesions Produced by the Filtrates*—The gross lesions produced by the filtrates involved mainly the joints with effusion of various gradations. The fluid was thin and clear in some and purulent in others. Edema of the soft tissues around the joints and the muscles with extension down into the tendon sheaths were found in some. Hemorrhagic effusion into the joints was noted in four cases.

*Pericardial Effusion* was found in 22 cases. The fluid was thin in some and purulent in others with variations in their consistency. Small, minute, and white patches near the apex were noted in some of the hearts. The valves showed no involvement on either side.

The *livers* were intensely congested and somewhat soft. When sectioned, the cut surface bulged and the edges curled. In some cases coecal infections were found which looked like abscesses, while others showed marked degenerative changes.

The *kidneys* were congested and softened. On section, cloudy swelling was observed with varying degrees of severity.

The *eyes* showed congestion and edema of the upper pole. In some pericorneal congestion which lasted from thirty-six hours to forty-eight hours was noted. The conjunctivae showed congestion and edema. Discharges were found in some cases and none in others. The iris in some cases showed congestion when examined under good and clear light.

The *spleen, stomach, and intestines* showed no lesions but were passively congested. Ascites was found in 12 cases with various stages of effusion.

Other lesions noted were dilatation of the heart in 2 cases, iritis in 1 case, congested kidneys in 4 cases, and congested livers in 2 cases.

The filtrates were fatal in twenty-four hours in 1 case, four days in 1 case, five days in 1 case, and six days in 2 cases.

*Microscopic Lesions Produced by the Filtrates*—The histologic studies made on the joints showed congestion and some round cell infiltrations. The synovial membrane in most cases showed little or no involvement. In the hearts, the myocardial vessels were congested. A rather diffuse degeneration of the cells was noted, evidenced by their granular cytoplasm with considerable disintegration. In marked cases necrosis and hyaline degenerative changes were found. The essential feature was the amount of damage done to the cells mainly due to the direct action of the filtrates. In two cases some fibrinous exudate, on the epicardial surface and infiltrations of round cells, some polymorphonuclear leucocytes and few plasma cells were noticed.

The *livers* showed marked congestion of the vessels. Extravasated red blood cells were seen scattered in some of the sinuses. The degenerative changes noticed were marked and diffuse. A great deal of necrosis and dis-

integration of the cells were seen, as the majority were very granular and vacuolated. Scattered about irregularly are some dark brown pigment granules where in some cases are taken up by the few leucocytes and phagocytic cells present. Minute foci of round cell infiltrations were found in some of the sections. The biliary passages showed hyaline degenerative changes and the walls were usually infiltrated with some round cells and few plasma cells. Coccidial infections were noted in seven cases.

The *kidneys* were markedly congested and the vessel walls were not thickened. The glomeruli and Bowman's capsules showed little or no involvement. The lumens of the convoluted tubules and the ascending limbs of Henle were reduced in size and sometimes obliterated. The epithelial lining showed cloudy swelling, fatty degeneration, or the two changes combined were present in some. In some cases the cells were necrotic and poorly stained. Desquamated and degenerated cells were sometimes found lying in the lumens. At other times extravasated red blood corpuscles and a few leucocytes were noticed mixed with the cell-debris forming a sort of cast. The collecting tubules, however, were not involved as the lining epithelium were well stained and their outlines were clear with the lumen empty.

*Gross Lesions Produced by the Organisms*—The gross lesions produced by the living organisms also showed joint involvements. They occurred in the knee, shoulders, and elbows. These were congested, swollen, and bulged with the fluid in the cavity held under tension. The fluid was cloudy in some and purulent in others with various gradations. The periaricular tissues were involved and extended into the soft tissues and exudate found along the tendon sheaths. Hemorrhagic effusion was noted in 4 cases.

The *pericardium* showed effusion of varying degrees and was purulent in 10 cases. Some of the hearts showed exudations on the surface. The myocardium when sectioned showed a greyish streak. In one case, endocarditis involving the valves was found.

The *livers* were intensely congested and softened. Miliary abscesses were found under the capsule and scattered irregularly in the organ. Coccidial infections were noted in some cases resembling abscesses.

The *kidneys* were congested and somewhat soft. Petechial hemorrhages and minute scars resembling infarction scars were noted. In one case miliary abscesses found in the cortex and medulla as well. Ascites was present in 13 cases showing gradations of effusion.

Other lesions noted were bursitis in 1 case, conjunctivitis in 1 case, and opacity of the cornea in 1 case.

Animals succumbing in twenty-four hours, 8 cases, in forty-eight hours, 1 case, in four days, 1 case, in five days, 1 case, in six days, 3 cases, and seven days, 1 case.

*Microscopic Lesions Produced by the Organisms*—The microscopic sections gave variable pictures. The *joints* showed congestion and infiltrations of some polymorphonuclear leucocytes and few lymphocytes. In few cases the synovial membranes showed some thickening and others slight necrotic changes superficially. The *hearts* showed congestion of the myocardial and epicardial

vessels. Fibrinous exudate with round cell infiltrations were noticed in some sections. The myocardium in a majority of the cases showed degeneration of the fibers. Military foci of necrosis were noticed in some cases where these necrotic changes were centrally located. In some of the foci was a zone of round cell infiltration surrounding the necrotic center composed mainly of lymphocytes and some plasma cells. In a few of the larger foci few organisms were found. They are in a majority of the cases, however, not generally present.

The *livers* showed congestion of the vessels. The cell columns were wider than usual due to the swelling of the individual cells. The swollen cells were rounded more or less and no longer polygonal in shape. The nuclei are pale and indistinct. The cytoplasm are granular and showed disintegration and small clear spaces were noticed in some. Military foci of round cell infiltrations were noted in some sections. In some larger foci showing necrotic changes feebly stained masses of cells were noticed surrounded by the liver tissue. The bile ducts in some showed round cell infiltrations. In few cases of marked hyperplasia of the biliary passages coccidial infections were noticed.

The *kidneys* showed congestion of the vessels. The convoluted tubules and the ascending limbs of Henle were mainly involved. The lumens were reduced in size. The lining epithelial cells were swollen and separated from each other due to loosening of the cement substance between them. The nuclei were indistinct in most of the cases. The cytoplasm showed degeneration and were granular. The shapes of the cells were irregular and their free margins in the lumen ragged. In some of the tubules there were accumulations of desquamated or possibly degenerated cells noted. The collecting tubules were not involved. The glomeruli appeared fairly normal and oftentimes are congested. Few emigrated leucocytes may be seen here and there lying in relation to the vessels. Military abscesses were noted in one case with inflammation of the pelvis. Infarcts in the cortex were noticed in one or two cases.

The sources of the organisms employed and the results of these rabbit inoculation tests with the living cultures and sterile filtrates are briefly summarized in Table I and show at a glance the types of lesions produced.

It will be noted that synovial and pericardial lesions occurred most frequently and their severity or degree is indicated by + and ++, signs for brevity and convenience.

The lesions found in then order of frequency and the percentages were as follows:

#### LESIONS PRODUCED BY THE FILTRATES

Synovitis (grade +)	31 cases	70.7%
Pericardial effusion (grade +)	21 cases	47.7%
Ascites	12 cases	27.2%
Abscess of the liver	9 cases	20.4%
Synovitis (grade ++)	6 cases	13%
Congestion of the kidneys	4 cases	9%
Hemorrhagic synovitis (grade -)	4 cases	9%

TABLE I

NO	CLINICAL DIAGNOSES	PARTS CULTURED	ORGANISMS FOLLOWED	RESULTS OF BACTERI INOCULATIONS	
				WITH CULTURES	WITH FILTERATES
1	1 Neuritis left arm	Tonsils	Streptococcus vir- idans	No 1a, Pericardial effusion +	No 5a, Pericardial effusion +
	2 Osteoarthritis			b, Synovitis +	b, Synovitis +
	3 Dental sepsis			No 2, Synovitis +	No 6a, Synovitis +
	4 Chronic tonsillitis				
2	1 Chronic nephritis	1 Tonsils 2 Lt ethmoid 3 Left sphenoid	Nonhemolytic streptococci	No 7a, Pericardial effusion +	No 9a, Pericardial effusion +
	2 Chronic otitis media			b, Synovitis +	b, Synovitis +
	3 Chronic otitis media			No 8a, Pericardial effusion +	c, Congestion of kidneys
	4 Chronic tonsillitis			b, Hemorrhagic synovitis +	No 10a, Pericardial effusion + b, Synovitis +
3	1 Secondary aneurysm	1 Teeth 2 Tonsils	Streptococcus vir- idans	No 13a, Pericardial effusion ++	No 15a, Dilatation of heart
	2 Myocarditis			b, Synovitis +	b, Synovitis +
	3 Dental sepsis			No 11a, Pericardial effusion +	No 16a, Dilatation of heart
	4 Chronic tonsillitis			b, Synovitis ++	b, Synovitis + c, Abscess of liver ?
4	1 Nephritis hemorrhagica	Tonsils	Streptococcus vir- idans	No 17a, Synovitis ++	No 19a, Iritis
	2 Chronic tonsillitis			No 18a, Pericardial effusion +	b, Pericardial effusion +
				b, Synovitis ++	c, Synovitis +
					d, Congestion of kidneys
5	1 Recurrent sacroiliac ar- thritis (Duration two years)	Tonsils	Nonhemolytic streptococci	No 21a, Pericardial effusion ++	No 23a, Synovitis +
	2 Chronic sore throats			b, Synovitis ++	b, Pericardial effusion +
				No 22a, Pericardial effusion ++	No 24a, Synovitis +
				b, Synovitis +	
6	1 Rheumatic endocarditis	Tonsils	Nonhemolytic streptococci	No 25a, Pericardial effusion ++	No 27a, Synovitis ++
	2 Rheumatic arthritis			b, Synovitis ++	(Fore and hind legs)
	3 Chronic sore throats			c, Abscess of liver ?	No 28a, Synovitis (hind legs) +
				No 26a, Pericardial effusion +	b, Hemorrhagic synovitis (fore legs) +
7	1 Chronic rheumatic arthritis (left knee partially ankylosed)	Tonsils	Nonhemolytic streptococci	No 29a, Pericardial effusion +	No 31a, Pericardial effusion +
	2 Chronic tonsillitis			b, Synovitis +	b, Synovitis +
				No 30a, Pericardial effusion +	No 32a, Synovitis ++
				b, Synovitis + c, Abscess of liver	b, Abscess of liver

\*1 = Indicative of acute congestion with effusion of clear fluid but with no definite evidences of inflammation  
 +1 = Indicative of purulent exudates with acute inflammatory changes



TABLE I—Continued

NO	CLINICAL DIAGNOSES	PALTS CULTURED	ORGANISMS LIMITED	RESULTS OF BACTERIAL INOCULATIONS	
				WITH CULTURES	WITH FRIABLES
8	1 Acute iritis and conjunctivitis, right eye	Pus from right eye	Hemolytic streptococci	No 33a, Pericardial effusion + b, Synovitis + c, Abscess of liver	No 17, Synovitis + No 18, Synovitis +
	2 Diabetes			No 31, Synovitis +	No 19a, Synovitis + +
	3 Infected tonsils	Tonsils	Nonhemolytic	No 35, Synovitis + No 36a, Pericardial effusion + b, Synovitis + + c, Abscess of liver and kidneys	b, Abscess of liver c, Pericardial effusion + No 10a, Hemorrhagic synovitis + b, Pericardial effusion +
10	1 Rheumatic endocarditis	Tonsils	Nonhemolytic streptococci	No 11, Synovitis + No 12, Pericardial effusion +	No 13a, Pericardial effusion + + b, Synovitis + No 11a, Synovitis + b, Abscess of liver c, Pericardial effusion +
	2 Anemia			No 15a, Pericardial effusion + b, Hemorrhagic synovitis +	No 17a, Synovitis + b, Pericardial effusion +
	3 Chronic tonsillitis	Tonsils	Nonhemolytic streptococci	No 15An, Synovitis + b, Burstis + c, Pericardial effusion +	No 18, Synovitis +
11	1 Scintilla neuralgia (right)	Tonsils	Nonhemolytic streptococci	No 16, Synovitis + No 16An, Synovitis + b, Abscess of liver	No 17a, Synovitis + b, Pericardial effusion +
	2 Chronic sore throats			No 16a, Synovitis + b, Abscess of liver	No 18, Synovitis +
				No 40a, Synovitis + b, Multiple abscesses of liver	No 51, Synovitis + No 52a, Pericardial effusion +
12	1 Frequent colds, head aches	Tonsils	Nonhemolytic streptococci	No 50a, Pericardial effusion + + b, Synovitis + + c, Multiple abscesses of liver	b, Synovitis + c, Multiple abscesses of liver ? (Cocci in infection)
	2 Chronic tonsillitis			No 51a, Synovitis + b, Multiple abscesses of liver	No 55a, Pericardial effusion + b, Synovitis +
	3 Chronic sinusitis			No 51a, Synovitis + b, Multiple abscesses of liver	No 56a, Synovitis + b, Multiple abscesses of liver
13	1 Rheumatic arthritis (poly)	Tonsils	Nonhemolytic streptococci	No 51a, Synovitis + b, Multiple abscesses of liver	No 55a, Pericardial effusion + b, Synovitis +
	2 Secondary anemia			No 51a, Pericardial effusion + + b, Synovitis +	No 56a, Synovitis + b, Multiple abscesses of liver
	3 Chronic tonsillitis			No 51a, Synovitis + b, Multiple abscesses of liver	No 56a, Synovitis + b, Multiple abscesses of liver

TABLE I—Cont'd

NO	CLINICAL DIAGNOSES	PARTS CULTURED	ORGANISMS EMPLOYED	RESULTS OF RABBIT INOCULATIONS	
				WITH CULTURES	WITH FILTRATES
14	1 Subacute bacterial endocarditis	Blood cultures	Streptococcus viridans	No 57a, Synovitis + b, Multiple abscesses of liver	No 59a, Synovitis ++ b, Multiple abscesses of liver c, Lenticlelids
	2 Acute myositis			No 58a, Pericardial effusion + b, Synovitis + c, Multiple abscesses of liver	No 60, Synovitis + No 61a, Pericardial effusion + b, Hemorrhagic synovitis + c, Multiple abscesses of liver
15	1 Subacute rheumatic fever	Tonsils	Nonhemolytic streptococci	No 61a, Pericardial effusion + b, Hemorrhagic synovitis + c, Multiple abscesses of liver	No 61a, Pericardial effusion + b, Hemorrhagic synovitis + c, Multiple abscesses of liver
	2 Rheumatic arthritis			No 62a, Synovitis + b, Multiple abscesses of liver	No 62a, Synovitis + b, Hemorrhagic synovitis +
16	1 Rheumatic fever, early	Tonsils	Nonhemolytic streptococci	No 65a, Pericardial effusion + b, Synovitis +	No 67, Synovitis + No 68, Synovitis +
	2 Rheumatic arthritis			No 66a, Pericardial effusion + b, Synovitis + c, Abscesses of lungs	No 67, Synovitis + No 68, Synovitis +
17	1 Bacterial endocarditis	Blood cultures	Streptococcus viridans	No 69a, Pericardial effusion + b, Synovitis ++ c, Multiple abscesses of liver	No 71, Congestion of liver and kidneys No 72, Synovitis +
	2 Bronchitis			No 70a, Pericardial effusion ++ b, Synovitis +	No 71, Congestion of liver and kidneys No 72, Synovitis +
18	1 Subacute bacterial endocarditis	Blood cultures	Streptococcus viridans	No 73a, Pericardial effusion + b, Hemorrhagic synovitis + c, Conjunctivitis	No 75a, Pericardial effusion + b, Synovitis ++ c, Multiple abscesses of liver
	2 Rheumatic arthritis (poly)			No 74a, Opacity of cornea b, Pericardial effusion + c, Synovitis +	No 76a, Pericardial effusion + b, Synovitis +
19	1 Frequent headaches	Tonsils	Nonhemolytic streptococcus	No 77a, Pericardial effusion + b, Synovitis + c, Abscess of liver	No 79a, Pericardial effusion + b, Synovitis + No 80a, Pericardial effusion +
	2 Infected tonsils			No 78a, Pericardial effusion + b, Synovitis +	No 80a, Pericardial effusion + b, Synovitis +

TABLE I—CONT'D

NO	CLINICAL DIAGNOSES	PARTS CULTURED	ORGANISMS EMPLOYED	RESULTS OF BACTERIOCOULTURES	
				WITH CULTURES	WITH INTRATHY
20	1 Inflammatory rheumatism	Tonsils	Streptococcus viridans	No 81b, Synovitis +	No 83, Congestion of liver and kidneys
	2 Infected and hypertrophied tonsils			No 82a, Synovitis ++ b, Pericardial effusion + c, Multiple abscesses of liver	No 84, Synovitis +
21	1 Migratory rheumatic fever	Blood cultures	Streptococcus viridans	No 85a, Pericardial effusion ++ b, Synovitis ++	No 87a, Pericardial effusion + b, Synovitis + c, Multiple abscesses of liver
	2 Subacute bacterial endocarditis			No 86b, Pericardial effusion ++ b, Endocarditis c, Synovitis ++	No 88a, Pericardial effusion + b, Synovitis + c, Abscess of liver
A	1 Immune external auditory canal, left	Pus from the auditory canal	Staphylococcus aureus	No 89a, Dilatation of heart b, Hemorrhage around joints c, Congestion of liver and kidneys	No 91a, Pericardial effusion + b, Synovitis + c, Abscess of liver and kidneys
				No 90a, Dilatation of heart b, Congestion of liver and kidneys	No 92a, Pericardial effusion + b, Dilatation of heart c, Congestion of liver and kidneys
B	1 Chronic suppurative maxillary sinusitis	Pus from maxillary sinus	Staphylococcus aureus	No 97a, Pericardial effusion + b, Synovitis ++ c, Abscess of liver and kidneys	No 99a, Pericardial effusion ++ b, Endocarditis c, Synovitis ++ d, Abscess of kidneys and liver
				No 98a, Dilatation of heart b, Pericardial effusion + c, Synovitis +	No 100b, Pleural effusion ++ b, Pericardial effusion + c, Endocarditis

### LESIONS PRODUCED BY THE ORGANISMS

Synovitis (grade +)	23 cases	50.2%
Pericardial effusion (grade +)	23 cases	50.2%
Synovitis (grade ++)	16 cases	36.3%
Abscesses of the liver	15 cases	30.4%
Ascites	13 cases	29.9%
Pericardial effusion (grade ++)	10 cases	22.7%

From the above it will be noted that the lesions shown by the two series of rabbits were quite similar in many instances. These, when studied histologically, were likewise similar in some instances in the microscopic changes produced. In general terms those produced by the filtrates showed greater damage to the cells and the tissues than those produced by the organisms. The pathologic changes found, however, showed varying degrees of inflammatory reactions which were dependent upon the toxicity of the filtrates or to the tissue resistance or to both factors, *but never showing any tendency to specific tissue localization* in the case of either filtrates or organisms.

In the case of the filtrates it would appear that pathologic tissue changes due to embolism may be definitely excluded, although this factor may have been operative in the production of lesions by intravenous injections of the organisms themselves. The general results of the study indicated that *filtrates, presumably carrying filterable toxins, are capable of producing lesions similar in kind and distribution to those produced by the living organisms plus such amounts of toxins as were produced during an incubation period of eighteen to twenty-four hours at 37° C*.

Furthermore, there were *no evidences of elective localization on the part of either the filtrates or the organisms*. Both produced synovial and pericardial changes as the usual or predominate tissue changes irrespective of the source of the streptococci and staphylococci and irrespective of the primary and secondary lesions presented by the patients from whom they were obtained. What other lesions developed in rabbits appeared to have no relationship to the source of the organisms employed.

### SUMMARY

1 Streptococci and staphylococci were recovered from presumably primary foci in twenty-one cases of chronic focal infections.

2 Sterile filtrates of broth cultures and young whole cultures of each were injected intravenously into rabbits.

3 The lesions produced by both were frequently similar in degree and distribution.

4 Synovial and pericardial changes were most frequently produced.

5 There were no evidences of elective or selective localization on the part of either filtrates or whole organisms in the rabbits of either series.

6 It would appear that toxins produced in the primary foci of focal infection may be absorbed and produce the secondary lesions.

7 On the basis of these results it is suggested that the early secondary lesions of focal infection may be due to these toxins and that the drainage

or extirpation of primary foci may afford prompt therapeutic results. In long established chronic secondary infections, however, the organisms themselves may be productive of the lesions and therefore probably independent of the primary foci and accounting for the absence of therapeutic improvement following their drainage or extirpation.

I am indebted to Dr. John A. Kolmer for suggesting and guiding this investigation and to Dr. Eugene A. Case for assistance in the pathological phases.

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## DIABETIC COMA OCCURRING NINETEEN TIMES IN THE LIFE OF A PATIENT WITH DIABETES MELLITUS\*

### CASE REPORT WITH AUTOPSY FINDINGS

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THE occurrence of diabetic coma in the course of diabetes mellitus is a complication to be expected and feared. The successful treatment of the diabetic patient in coma depends on the previous duration of the comatose state and on the exciting cause of the coma. It is not unusual during the course of the life of the diabetic patient to have him go into coma once or twice, but it is rather extraordinary for a patient to have been in diabetic coma and to have recovered from it on nineteen occasions.

The patient in question was first admitted to the Pediatric Service of Bellevue Hospital in November, 1925, with the usual symptoms of diabetes and a history of drowsiness for a period of two weeks prior to admission. The diabetes had apparently started three months previously. On the first admission the child was ten years old. She appeared drowsy and there was a distinct odor of acetone to the breath. The skin was dry. The temperature was 100.4° F., and the pulse rate was 96. The weight was forty-nine pounds. The urine showed complete reduction with Benedict's solution and the acetone and diacetic acid were four plus. The blood sugar was 970 mg., and the O<sub>2</sub> combining power was 15 volumes per cent. There was an infec-

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tion of the middle ear, which opened spontaneously, and an abscess of the left arm. She received in the course of treatment 750 cc of fluid by mouth, 2200 cc by enema, 425 cc by infusion. The urine output during this time, a period of forty-four hours, was 780 cc. The blood sugar fell to 450 mg, and the  $\text{CO}_2$  rose to 20 volumes per cent.

From the year 1925 until October, 1929, the patient was admitted twelve times to the pediatric service. Two of these admissions were in insulin shock. Of the other ten admissions, seven were in severe diabetic coma and three were in a profound state of ketosis veiling on diabetic coma. Table I is a summary of these twelve admissions. It was characteristic, in this case, that on every occasion the onset of coma was accompanied by abdominal pain, which was generalized, abdominal tenderness, and vomiting. These symptoms were also present on the next nine admissions, Table II, when the patient was admitted to the Third (New York University) Medical Division, Bellevue Hospital. In each instance, as soon as the ketosis was overcome these symptoms and signs would disappear and during the periods when the patient's diabetes was controlled no abdominal tenderness could be elicited. The question of a chronic appendicitis was naturally considered, but the diagnosis did not seem warranted by the findings when the patient was sugar free. No mention was made of any abnormal findings in the appendix on postmortem examination.

We have not attempted to report the urine and blood findings of each admission. They would make a volume by themselves. The admission blood sugars were never below 330 mg, and the  $\text{CO}_2$  combining power of the plasma varied from 5 volumes per cent to 40 volumes per cent. The urine usually showed albumin and casts during the period of ketosis, as well as sugar and the acetone bodies.

The amounts of insulin required to overcome the comatose state varied from 200 units to 530 units.

The patient's final attack of diabetic coma was treated in the New York Hospital and is not included in Table II. We are indebted to the New York Hospital for their findings, the report of the treatment and the complete autopsy report.

#### REPORT FROM NEW YORK HOSPITAL

M. S., aged sixteen years, was a patient in this hospital from December 3, 1931 to December 4, 1931, when she died. She was admitted in a state of coma at 9:45 AM. On December 2, she felt thirsty and vomited. That evening at 11:30 PM she became unconscious and was brought to this hospital. Patient was found in a state of restless coma with very flushed cheeks, Kussmaul breathing, soft eyeballs, dry mouth, and fruity odor on breath. The skin was very cold, temperature  $94.2^\circ \text{F}$ .

*Progress*—Urine showed four plus sugar, four plus acetone, and diacetic acid. Insulin was given at the rate of about 25 units an hour. Glucose enema was given on admission, 100 grams being instilled. As the enema was not satisfactorily absorbed, a needle was put into the vein and a continuous saline infusion started which was allowed to run steadily, administering 500 cc an hour until  $4\frac{1}{2}$  liters had been absorbed. By this time the mouth and lips, which had been extremely dry, were somewhat moist and the pulse, which had been very weak and thready, had very markedly improved in quality. The temperature, however,

had shot vertically upward from 94.2° to 99.6° to 105.0° F. Urine specimens taken at hourly intervals during the day showed continuously four plus sugar, acetone, and diacetic acid. At about 11:30 A.M. her pulse became somewhat irregular, respirations catching and halting. Urine specimen at this time showed very marked diminution in acetone and diacetic acid with slight diminution of sugar. Patient expired at 12:50 A.M., December 4, 1931. Autopsy was performed.

*Diagnosis*—Diabetes mellitus

*Complications*—Acidosis, diabetic bronchopneumonia (hemorrhagic)

*Postmortem Examination*—Kidneys. Each weighed 150 grams. The capsules stripped readily leaving a smooth surface. The surfaces presented an unusually regular mass of red strands which separated grayish areas of 0.1 cm. in diameter from one another. On section, the markings seemed regular but they were very distinct, there being a very marked contrast between the gray areas and the injected ones. Pelvis and ureters were uninjected.

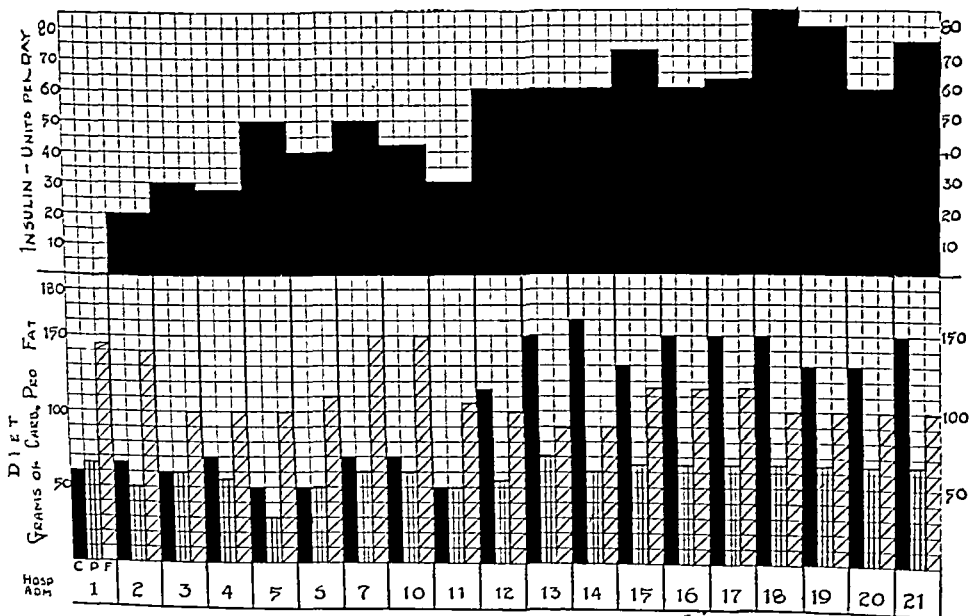


Chart I.—Showing carbohydrate, protein and fat content of diet and units of insulin after each hospital admission

Microscopically, there was marked granular degeneration, presumably in part post mortem. There was marked spotty congestion. There was a small scar like area of uncertain nature.

*Pancreas*. Seemed to be of about normal size, but was soft, slippery and somewhat yellowish throughout. The ducts were not grossly recognized. It weighed 100 grams.

Sections from the head and body showed so much postmortem change as to make their study useless. That which probably came from the tail was partially preserved and showed a single moderately hyperplastic island of Langerhans. The perilobular tissue showed marked postmortem change.

*Spleen*. Was soft and small, weighing only 100 grams. The capsule was of the normal purplish color. On section, the pulp was dark red in color and very soft solid in consistence. It seemed to form semi isolated masses of about 0.3 cm. in diameter. Two firm but not apparently calcine yellowish nodules, one 0.1, one 0.2 cm. in diameter, were found in the pulp.

TABLE I

NO	ADMISSION	DISCHARGE	SYMPTOMS ON ADMISSION	DURATION OF SYMPTOMS PRIOR TO ADMISSION	POSITIVE PHYSICAL FINDINGS	T	I	R	B I	W F	DIPLOMATIC CHARGE					INSULIN			W T	W B C	REMARKS
											O	P	F	Bk.	LU	SU					
1	11/12/25	3/ 8/26	Polyuria, Polydipsia, Polyphagia, Loss of weight (6 lb in 3 mo), Drowsy for 2 weeks	3 months	Acetone breath, Drowsy	100 4° T	96	22	80/40	19	60	65	145	0	0	0	56		10,100 Polys 44%		
2	5/18/26	6/20/26	Dietary adjustment	Glycosuria for 7 days	Urine Sugar 4+ Acetone 4+	100 4° T	128	28	N	52	65	50	140	X	0	X	52				
3	9/26/26	1/ 9/27	Abdominal pain, Vomiting	3 days	Dry skin, Comatose, Acetone breath Urine Sugar 4+ Acetone 4+	97 0° T	116	36	100/80		60	60	100	IX	IX	IX					
4	1/18/27	6/ 4/27	Vomiting, Drowsiness	18 hours	Dry skin, Drowsy Urine Sugar 4+ Acetone 4+	98 6° T	120	24	70/50		70	55	100	XX	XIV	XVI			8,100 Polys 71%	Throat culture K L Bacilli, antitoxin given	



TABLE I—CONT'D

NO	ADMISSION	DISCHARGE	SYMPTOMS ON ADMISSION	DURATION OF SYMPTOMS PRIOR TO ADMISSION	POSITIVE PHYSICAL FINDINGS	T	P	R	B P	WT	DIET ON DISCHARGE				INSULIN			WT W B C	REMARKS
											C	I	1	2	Bk	IU	SU		
5	10/18/27	10/27/27	Abdominal pain, Vomiting, Ate excess fruit	24 hours	Abdomen distended No tenderness Urine Sugar 4+ Acetone 4+ 10/19/27 Tenderness and rigidity in R L Q 10/20/27 Urine Casts and RBC, Pyelitis (?)	99.0° F	100	26	N		40	130	100	100	100	100	100	27,000 Polys 87%	
6	11/1/27	1/11/28	Drowsiness, Sem coma	24 hours	Urine Sugar 4+ Acetone 4+	99.8° F	108	28	100/74		40	50	105	105	105	105	105		Abcess of right arm
7	5/29/28	6/17/28	Epigastric pain, Vomiting, Drowsiness	12 hours	General abdominal tenderness Urine Sugar 4+ Acetone 4+	100.8° F	120	28	88/36		60	60	154	154	154	154	154		Omitted night dose of insulin
8	6/19/28	6/23/28	Insulin shock			96.0° F	74	16	102/78										
9	6/26/28	6/27/28	Insulin shock		Blood sugar 30	97.4° F	80	26	N		60	60	164	164	164	164	164		
10	12/27/28	12/31/28	Impending coma			98.0° F	90	24			60	60	154	154	154	154	154		
11	7/29/29	8/5/29	Abcess of the right thigh	7 days	Urine Sugar 4+ Acetone 4+ Diabetic acid 4+	98.4° F	114	20			40	50	108	108	108	108	108		
12	10/14/29	10/26/29	Pain in left lower quadrant	?	Slight tenderness in left lower quadrant	100.2° F	126	22			115	56	102	102	102	102	102	10,600 Polys 71%	

TABLE II

NO	ADMISSION	DISCHARGE	SYMPTOMS ON ADMISSION	DURATION OF SYMPTOMS PRIOR TO ADMISSION	POSITIVE PHYSICAL FINDINGS	T	R	B P	WT	DIET ON DISCHARGE			INSULIN			WT	W B C			REMARKS
										O	I	F	Bk	LU	SU					
13	12/10/29	12/23/29	Abdominal pain marked Vomiting	12 hours	Acutely ill Flushed Eyes negative General abdominal tenderness This decreased but did not disappear	98.6° 120 F	24	120/68	80	150	70	90	XX	XX	XX	80.5	20,000 Polys 89%			
14	2/17/30	3/23/30	Infection of left thigh, Vomiting, Abdominal pain	4 days 3 days	Dehydrated Erythema soft Acetone breath +, Abdominal tenderness Cellulitis of thigh	99.0° 140 F	24	-----	85	160	60	90	XXXV	X	XV	87.5	12,600 Polys 70%			Incision of abscess of left thigh
15	5/22/30	5/29/30	Nausea and vomiting, Abdominal pain	24 hours	Acetone breath + Drowsy Dehydrated Epigastric tenderness Abdomen soft	99.0° 140 F	22	-----	83	140	65	115	XXX	XVIII	XV	88.0	20,000 Polys 82%			
16	6/26/30	7/8/30	Vomiting, Epigastric pain	12 hours	Acetone breath + Dehydrated Erythema Kussmaul breathing Tenderness in RUQ	99.4° 148 F	28	90/50	87	150	65	115	XXX	X	XX	87.0	20,700 Polys 80%			

TABLE 11—Continued

NO	ADMISSION	DISCHARGE	SYMPTOMS ON ADMISSION	DURATION OF SYMPTOMS PRIOR TO ADMISSION	POSTMORTEM FINDINGS	T	I	I	BP	WF	DISCHARGE			INSECTA			WBC FINDINGS	
											C	I	P	BK	II	SL	W	HGB FINDINGS
17	8/ 1/40	8/14/30	Vomiting, Abdominal pain	12 hours	Acetone breath skin Eyeballs soft Tenderness in RUQ	100.6° F	120	22	120/70	90	150	65	115	XXX	XII	XX	910	13,200 Polys 85%
18	1/ 7/31	1/25/31	Vomiting, Abdominal pain	24 hours	Acetone breath dratted Eyeballs soft Drowsy Abdomen distended Tenderness in RUQ	97.5° F	102	40	-----	98	150	61	100	XL	VI	XXX	1010	13,600 Polys 85%
19	5/23/31	6/ 1/31	Vomiting, Nausea	7 days	Acetone breath dratted Eyeballs soft Tongue beefy Drowsy Hy peruric No abdominal tenderness	99.6° F	124	36	120/55	102	140	64	100	XXXX	XX	XXX	1025	23,900 Polys 79%
20	6/11/31	7/18/31	Vomiting, Abdominal tenderness Abscess of thigh	1 day 10 days	Acetone breath dratted Eyeballs soft Drowsy Hy peruric Infected left thigh	99.2° F	110	10	110/35	---	140	61	100	XXXX	0	XXX	---	15,400 Polys 75%
21	9/ 2/31	9/18/31	Vomiting, Abdominal pain	21 hours	Acetone breath dratted Eyeballs soft Drowsy Abdominal tenderness	99.0° F	120	20	116/0	103	150	61	100	XL	V	XXX	1030	11,600 Polys 52%

Microscopically, the separate (yellowish) nodules showed caseous degeneration in their centers with peripheral fibrosis. The picture was rather suggestive but not diagnostic of tuberculosis. The pulp showed great postmortem change. Lymphocytes were scanty except in the malpighian bodies.

*Anatomic Diagnosis*—Necrosis of pancreas, postmortem (diabetes mellitus, from clinical history), chronic bronchopneumonia, hemorrhages under pericardium and pleura, possible old tubercles of spleen and kidney.

*Bacteriologic Findings*—Aerobic and anaerobic cultures of blood from heart show *B. Welchii*.

*Probable Cause of Death*—Diabetes mellitus.

## DISCUSSION

There is no doubt that in this case the severity of the diabetes was increased by the constant dietary indiscretions, resulting in the repeated occurrence of coma or profound ketosis. In Chart I we have endeavored to show graphically the increasing insulin requirement over the seven year period of the patient's life. The diet during this time changed from a high fat low carbohydrate diet, to a lower fat high carbohydrate diet, although neither of these constituents of the diet reached the levels that are being used today. Until October, 1929, the patient's carbohydrate intake varied from 40 to 60 grams and the fat intake varied from 100 to 150 grams. The insulin during this period rose steadily from 20 units a day to a peak of 50 units a day. During the next period, when the carbohydrate was raised until it finally reached a level of 150 grams daily, the fat was kept at about 100 grams daily. The insulin requirement rose from a total daily dose of 60 units to a peak of 85 units. These diets represent what the patient actually was able to tolerate by the end of each period of hospitalization. They were never really continued long enough for one to make any statement as to the comparative effects of the two types of diet as the patient never adhered to her diet when out of the hospital.

The degree of dehydration that was present on almost every admission is borne out by the small urine volume as compared to the large fluid intake. The amounts of fluid retained on the last nine admissions varied from 2700 cc to 8300 cc. On six of these last nine admissions (Nos. 13, 14, 16, 18, 19, 20) anuria was present and was only overcome by the use of continuous intravenous infusions. Functional renal insufficiency as a cause of death in diabetic coma has been stressed by Coburn.<sup>1</sup> The occurrence of anuria was always accompanied by a fall of blood pressure. When it was possible to raise this by infusions or transfusions, urine excretion was resumed, there was an increased excretion of acetone bodies and recovery took place. In Coburn's cases death occurred in sixteen patients (18 per cent) following renal shut-down.

The other striking feature in this case was the presence on each admission of vomiting, abdominal pain and tenderness. This occurred with the utmost regularity. Joslin<sup>2</sup> has drawn attention to this complication of diabetic coma and considers it as a condition which may confuse the diagnosis. Unfortunately, the leucocyte count is not much of an aid in differentiating coma from an acute abdomen, as leukocytosis is usually present in coma. Joslin.<sup>3</sup>

The surprising thing, in the case of this patient, was that she withstood so many attacks of coma. But for insulin she would undoubtedly have succumbed to the first or second attack.

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## BLOOD REGENERATION IN DOGS AS INFLUENCED BY LIVER AND IRON PREPARATIONS<sup>2</sup>

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THIS work was undertaken to ascertain the blood regenerating properties of two fractions of horse-liver extract, one of which is known to be active in pernicious anemia and the other (an aqueous extract of whole liver) is presumed to be active in secondary anemia on the basis of the work of Whipple and others.<sup>1</sup> The effectiveness of iron in various forms was also studied.

## METHODS

In order to obtain comparable results, the experiments were performed on three litters of dogs, whose past history was known and whose diet had been controlled since birth. The experiments were divided into groups, a litter representing one group at a time. When a litter was to be used over again, several months were allowed to elapse between the two tests. In all the experiments, the animals were kept on a basal diet and in each group, one dog used as a control. This diet was low in iron but permitted blood regeneration in about thirty-five days. The constituents of the diet were: white bread 73 gm., bran 5 gm., dried skim milk 20 gm., canned tomatoes 15 c.c., cod liver oil 0.7 c.c., yeast 1 gm., Osborne and Mendel's salt mixture without iron 0.6 gm. and horse fat 7 c.c. The dogs remained in excellent health and consumed their ration completely each day. The substances to be investigated were mixed with the food.

Anemia was produced by bleeding. The blood volumes were first determined, then from one quarter to one-third of the total volume of blood was drawn each second or third day until the hemoglobin value fell between 30 and 45 per cent. It required fewer bleedings to produce anemia the second time, due probably to depletion of iron reserve. When the hemoglobin was

<sup>2</sup>From the Laboratory of the Chappell Foundation for Organo-therapeutic Research, Rockford, Ill.

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TABLE I

GROUP I		AV WEIGHT	DAY	1st	79	11 13	15	18 20	21 24	25 28	30 32	35 36	39	45	INCREASE OF 40% HGL IN DAYS	INCREASE OF GY HGL IN 10 DAYS, % PER DAY	CALCD IN % OF END VALUE	INCREASE OF GY HGL IN 20 DAYS, % PER DAY	CALCD IN % OF END VALUE	GAPD IN % OF END VALUE	AV COLOR INDEX
1	No 29 Control	13	RBC % Hgl %	3 35	4 46	53 60		65 69	70 80	71 81	73 89	80 95			12	42	22	35	22	18	055
2	No 30 44 mg I <sub>2</sub> as neutr glycerol FeCl <sub>3</sub>	11 1	RBC % Hgl %	38 46	50 64	72 96		83 96	83 106	72 100					10	88	41	60	41	30	065
3	No 31 35 cc L <sub>av</sub> Ext P 8 1	12	RBC % Hgl %	37 29	35 30	48 43		59 49	61 49	64 54	74 57	84 70	94 72	10	35	16	095	21	095	11	042
4	No 32 50 cc defibr Blood	14	RBC % Hgl %	31 40	50 48	73 82	73 89	86 89		84 87	74 92	73 86	84 100		10	86	37	58	37	25	056
5	No 33 44 mg Fe as acid glycerol FeCl <sub>3</sub>	13	RBC % Hgl %	33 48	65 67	67 69		66 77	64 85	71 93	72 87	68 85	71 87		23	57	31	37	31	21	060

TABLE II

GROUP 2	AV WT KG	Day	1	46	79	10 12	13 15	16 18	19 21	22 24	23 27	28 30	31 33	34 36	1 CLASS OF 40% HGT IN DAYS	IN DAYS 10				IN 1/2 OF END VITL	IN 1/2 OF END VITL	IN 1/2 OF END VITL	AV COLOR
																IN 1/2 OF END VITL	IN 1/2 OF END VITL	IN 1/2 OF END VITL	IN 1/2 OF END VITL				
6 No 31 Control	7	RBC	11	1	15	51	61		69	70	70				21	15	15	20	15	15	20	26	0 57
7 No 36 15 cc Lys EN 5 1 1	79	RBC	19	15	50	65	70	73	81	72	85				16	11	14	15	14	14	15	30	0 57
8 No 35 110 mg FeO as acid glycerol FeCl <sub>3</sub>	7	Hgt %	39	52	65		77	50		81	73				17	11	12	21	23	23	26	0 60	
9 No 37 110 mg FeO as neutr glycerol FeCl <sub>3</sub>	79	RBC	25	17	11	60	60	71	69	64	87	65	70		11	3	27	11	27	27	25	0 61	
10 No 35 110 mg FeO as B L FeO mixture	75	RBC	12	18		51	65	70	81	88		57	87		11	16	11	15	11	11	12	0 61	
11 No 39 50 cc Blood, * after 25th day L B FeO mixture	7	Hgt %	11	57	79		82	61		101		69			11	10	10	11	10	10	11	0 61	
		RBC	27	11	58	59	85	80	61	66	61*	69		70	11								
		Hgt %	17	51	70	77		85	80		85	85		80									

\*The normal values of No 39 are 77% Hgt 6.2 millions RBC

\*The normal values of No 39 are 77% Hgt 6.2 millions RBC





TABLE IV

TABLE IV																				
GROUP 4	AV WT KG	DAY	1	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	28-30	31-33	34-36	INCREASE OF 40% HGL IN DAYS	DAILY GAIN IN GRAMS OF HFMODIOLIN DURING FIRST				IN COLOR
																10 DAYS		20 DAYS		
																IN GR	IN % OF FINAL VALUE	IN GR	IN % OF FINAL VALUE	
16 No 35 Control	76	RBC Hgl %	33 34	48 37	58 51	63 57	63 69	63 61	66 59	61 59	61 59	70 76	70 82	78 81	25	22	20	2	18	0.50
17 No 36 145 mg Fe as aqueous Fe Cl <sub>2</sub>	80	RBC Hgl %	34 38	41 55	56 63	52 61	61 71	61 71	59 71	59 71	59 71	76 83	63 76	65 82	32	33	33	21	21	0.60
18 No 34 145 mg Fe as neutr glycol FeCl <sub>2</sub>	74	RBC Hgl %	30 32	48 54	56 69	60 73	66 80	63 83	68 83	68 83	68 83	69 83	70 83	65 83	11	47	11	31	32	0.60
19 No 37 110 mg Fe as B L Fe mixture	84	RBC Hgl %	35 37	49 49	65 70	65 84	55 76	55 83	67 83	72 84	72 84	80 91	80 91	71 91	10	57	16	10	32	0.60
20 No 39 110 mg Fe as B L Fe mixture	75	RBC Hgl %	25 32	40 55	45 61	57 69	57 69	76 87	68 87	70 86	70 86	69 86	69 86	69 86	11	47	10	43	37	0.63

GROUP 4

TABLE V

	GROUP 5	AV WEIGHT		1	5	10	15	20	25 DAYS	FINAL	INCREASE OF 40% HEMOGLOBIN IN DAYS	DAILY GAIN IN GRAMS OF HEMOGLOBIN DURING FIRST 20 DAYS				AV COLOR INDEX
												% OF FINAL VALUE				
												IN GM	IN GM	IN GM	IN GM	
21	No 32 Control	1175	RBC HgI	304 39	347 415	40 47	53 55	575 61	63 62	72 75	32	2	12	23	13	058
22	No 31 50 cc Lav Ext P 8 1	136	RBC HgI	24 29	32 31	43 33	465 15	50 47	515 53	92 75	29	11	075	22	15	051
23	No 29 50 cc Lav Ext P 8 1	106	RBC HgI	32 375	42 435	42 49	60 50	63 586	66 72	78 86	27	21	14	23	15	051
24	No 33 50 cc Lav Ext S 4 1	130	RBC HgI	28 41	35 17	505 60	55 65	64 83		68 93	19	45	21	60	26	062

TABLE VI

	GROUP 6	AV WEIGHT		1	5	10	15	20	25 DAYS	FINAL	INCREASE OF 40% HEMOGLOBIN IN DAYS	DAILY GAIN IN GRAMS OF HEMOGLOBIN DURING FIRST 20 DAYS				AV COLOR INDEX
												% OF FINAL VALUE				
												IN GM	IN GM	IN GM	IN GM	
25	No 40 Control	140	RBC Hgl	301 38	41 48	485 53	50 59	65 62		69 80	32	35	17	31	15	055
26	No 41 145 mg Fe as aqueous FeCl <sub>3</sub> soln	117	RBC Hgl	295 38	38 51	46 60	55 71	55 72	6 81	63 84	23	37	25	37	21	065
27	No 12 145 mg Fe as acid glycerol FeCl <sub>3</sub> soln	129	RBC Hgl	256 30	325 39	13 67	55 60	68 77		70 86	18	53	26	60	31	060
28	No 43 145 mg Fe as neutral glycerol FeCl <sub>3</sub> soln	141	RBC Hgl	305 375	44 615	515 656	63 77	63 84		66 86	15	70	35	61	32	061
29	No 44 110 mg Fe as L B Fe mixture	158	RBC Hgl	275 37	425 64	51 65	57 91	70 85		72 88	12	66	40	71	32	067

sufficiently reduced, the substances to be tested were added to the diet, while the control was maintained as before on the basal ration. Erythrocyte counts and hemoglobin determinations were made on an average of every fifth day. The Newcomer method was used for hemoglobin determinations, and the same disc, standardized by the Livshien method,\* was used throughout the experiment. The hemoglobin values are expressed in percentage, 16.92 gm per 100 cc being taken as the normal. Reticulocyte counts were made but omitted because they are of little significance in this connection. Blood volume determinations were made frequently, the method of Rowntree and Brown<sup>7</sup> being used. The total amount of hemoglobin in circulation was calculated from the blood volume and hemoglobin determination.

All of the hematogenetic substances tested were mixed with the food complete ingestion was obtained in every case. These substances were as follows:

*Preparation 1*—Liver extract P—a purified horse liver extract of known potency in pernicious anemia, described by Richter, Meyer and Ivy (1932).<sup>4</sup> One cubic centimeter of the extract represents the antipernicious anemia value of 8 gm of whole liver.

*Preparation 2*—An aqueous extract of horse liver, evaporated in vacuo so that one part of extract represents 4 parts of whole liver, this is referred to as Liver Extract S No. 4-1.

*Preparation 3*—An aqueous solution of ferrous chloride.

*Preparation 4*—Eighty grams of ferrous chloride were made up to 100 cc with water and filtered into 700 cc of glycerol. The reaction of this solution is decidedly acid.

*Preparation 5*—The fifth preparation was made from the preceding ferrous chloride solution in glycerol (No. 4) by treating it with 10 per cent sodium hydroxide until a  $P_H$  of 6.8 was attained as judged by the indicator method, using bromthymol blue as indicator. This value does not represent the exact  $P_H$ , since in an organic solvent the indicator method is subject to a correction. About 20 cc of 10 per cent sodium hydroxide are required for 800 cc of the Solution 4. On dilution with water ferrous hydroxide precipitates. The mixture is not highly stable and therefore was freshly prepared each week and was kept in small corked bottles.

*Preparation 6*—Since it was found that serum, presumably the serum proteins, stabilize the colloidal iron present in Preparation 5, a mixture was made of defibrinated blood and liver extract S 4-1 in equal parts. The neutralized Mixture 5 together with alcohol and sugar solution was added in such a proportion, that a third aliquot portion was added. The final mixture contained in 100 cc, 220 mg of iron element in form of Preparation 5 and 14½ per cent of alcohol. The rationale of this preparation has been discussed together with clinical reports by Richter, Meyer and Legere.<sup>5</sup>

\*I wish to express my thanks to Dr. Elvenjem University of Wisconsin for supplying me with the crystallized hemin used for this standardization.

## RESULTS

The findings are summarized in Tables I to VI, which compare the hemoglobin regeneration of control animals with the regeneration which occurred when the test materials, in the stated dosage, were added to the diet

## DISCUSSION

The small number of animals used for each substance, together with the difficulty of producing entirely comparable anemic states in the various animals, makes it difficult to draw more than tentative conclusions from these results. The most significant result is the apparent superiority of the neutralized glycerol-iron mixture (Preparation 5) over other forms of inorganic iron. It is hardly necessary to review the voluminous and discordant literature, which deals with the various forms of iron in secondary anemia, or to point out that iron, as it is usually administered in small doses, is not highly effective. Whipple<sup>6</sup> has shown that beef-liver feeding produces maximal regeneration of hemoglobin. Whole liver was not fed in our experiments, but it is significant that the neutralized glycerol-iron produces regeneration at about the same rate as an aqueous extract of whole liver, and not much less than a whole liver aqueous extract combined with the neutralized iron. It is difficult to explain this superiority. The reason for the use of the neutralized iron was solely the avoidance of irritation of the stomach. It may be something like the "half masked iron" of which Starkenstein<sup>7</sup> (1928) speaks, and which is supposed to be partly colloidal hydroxide and partly complex iron ions.

The triple mixture of liver, blood, and iron gave the results which might be expected, namely, a very rapid return to normal. One might even anticipate that this mixture would produce hemoglobin regeneration at a much more rapid rate than any form of iron given alone. However, the superiority is not marked, indicating that in these animals the anemia is due primarily to an iron deficiency. An interesting effect is noted in the animals in which liver fraction P 81 was used (antipermeious anemia fraction). Here the formation of stroma, as indicated by the red count, was increased beyond the normal. The apparent further decreased ability of the body to form hemoglobin, as might be concluded from Test 3, was not confirmed in Tests 22 and 23. It shows, however, the inefficacy of this fraction in secondary anemia when iron is missing. From the low iron content (only traces) of this fraction, more could not be expected.<sup>8</sup>

## SUMMARY

Experimental hemorrhagic anemia in dogs has been treated with the liver extract active in pernicious anemia, with an aqueous extract of whole liver and iron in various forms. The best results were obtained with a neutralized solution of ferrous chloride in glycerol, in which this iron compound was combined with aqueous whole liver extract and blood. Purified liver extract (fraction G, active in pernicious anemia) stimulated erythropoieses but

has no favorable effect on the hemoglobin formation. Our results indicate that iron, particularly in a 'colloidal form' plays an important, if not an essential role, in the regeneration of hemoglobin in hemorrhagic secondary anemia. Although we do not doubt that an aqueous extract of whole liver may contain organic substances which play a more or less specific rôle in the regeneration of hemoglobin in secondary anemia we believe that the copper and iron content of aqueous whole liver extracts are of significant importance.

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## BISMUTH IN THE TREATMENT OF SYPHILIS<sup>2</sup>

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THE extensive use of this drug in the treatment of syphilis dates from the work of Sazciac and Levaditi,<sup>1</sup> in 1921, but the experience of the first few years was gained exclusively with French "patent" and "proprietary" preparations which were very diverse in their composition. Even now, at the end of 1932, there is still so much to learn about bismuth that I offer the following as merely a tentative statement of its status

### METHOD OF ADMINISTRATION

This is simple: the drug is not effective when given by mouth, is very likely to cause severe reactions and even death when given intravenously, and therefore is administered safely and with good effect only intramuscularly. The preparation should be drawn into the syringe through a short thick needle which is then to be discarded for a longer and slenderer one with a clean lumen. Insert the needle to point down and slightly in from a point in the inner part of the upper outer quadrant of the buttock. After carefully aspirating (important!) to be sure that the point is not in a blood vessel, inject slowly and finish with a bubble of air to prevent drawing some bismuth out toward the surface as the needle is withdrawn, which might later cause a painful button of induration. Sometimes such buttons last for a week or more.

### SOLUBLE AND INSOLUBLE PREPARATIONS

Early attempts to compare the absorbability of aqueous solutions of soluble salts and suspensions of insoluble salts in oils by x-ray studies of intramuscularly injected compounds, were not successful, but the recent studies of numerous investigators (Hanzlik and Mehrtens,<sup>10</sup> von Oettingen,<sup>4</sup> Lomholt,<sup>3</sup> and others) of the rate of excretion in the urine and feces, coupled with accumulating clinical experience, have fixed the status of these two classes of preparations with reasonable certainty.

### WATER SOLUBLE SALTS

These preparations are more rapidly and more regularly absorbed than the oily suspensions, which is both an advantage and a disadvantage: advantageous, in that it enables quick results to be attained, so quick indeed that some observers feel that bismuth is challenging the place of arsphenamine in early syphilis (see below), disadvantageous, in that they must be administered twice or thrice in the week, and are thus made quite impracticable for routine clinic use and even for office practice except in unusual instances.

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Furthermore, these aqueous solutions are very apt to cause a troublesome stomatitis if they are pushed too rapidly. An advantage, on the other hand, is that there is no danger of embolism or infarction following these injections, which however, are much more painful than are those of the oily suspensions. The following are perhaps the best of the aqueous preparations, the dosage given is for a single course, which may be repeated one or more times after an intermission of one month.

a *Bismuth sodium tartrate* (N N R) —The initial dose is 0.015 gm ( $\frac{1}{4}$  gram), which is doubled usually with the second dose and continued in two or three doses weekly for six to ten weeks.

b *Bismosol* (N N R) —Administered usually twice a week in 1 cc doses until twenty doses have been given.

c *Thio Bismol* (N N R) —The average dose 0.2 gm ( $\frac{1}{2}$  gr) is injected twice weekly for a series of twelve to fifteen doses.

d *Iodobismutol* —This preparation is not really an aqueous solution but is included here because it contains an active bismuth salt dissolved in ethylene glycol and has for all practical purposes the above enumerated advantages and disadvantages of the solutions of salts in water. Ten injections of 2 cc each are given in about three weeks.

#### SUSPENSIONS IN OIL

These preparations are much more slowly and irregularly absorbed than the solutions and are not generally felt to be as well suited for treatment of early syphilis as are the latter. If this prolonged absorption were regular in rate the advantages would be obvious in late syphilis, but unfortunately during a period of very slow absorption a pocket of bismuth seems at times to be emptied into the circulation with a resultant attack of stomatitis of an explosive type. The injection of these suspensions causes much less pain than the injection of aqueous solutions, sometimes none at all, and the injections need be made only once a week. But to counterbalance these great advantages there are the following disadvantages: (a) sterile abscesses of rather large proportions may occur even a long time after the injections have been stopped, either they must be opened or they will spontaneously rupture, (b) though embolism has occurred with perhaps no greater frequency than in the use of insoluble mercury, infarction following injection into an artery has been noted in a rather distressingly large number of instances, there is immediately great pain and nearly always ultimately gangrene and sloughing out of the affected area. From the standpoint of clinic administration, there is another serious disadvantage of the oily suspensions, as shown by Cole, Henderson et al (1931)<sup>5</sup> syringefuls taken from oily bismuth suspensions in bulk will contain very variable amounts of the metal, this objection does not of course apply in office practice where individual dose ampuls are used.

The following are perhaps the best preparations:

a *Mesuro* (N N R, 1932) —The initial dose of  $\frac{1}{2}$  cc is increased to 1 cc at the second dose and injected eight to twelve times at intervals of one week.

b *Olio Bi-Roche* (N N R, 1932) —Weekly injections of 2 cc are given in a course of twelve to twenty.

*c Potassium Bismuth Tartrate (N N R, 1932)*—This preparation is unfortunately commercially available in a rather confusing form if used in bulk, 1 c c is injected once (or twice) weekly for twenty-four (or twelve) weeks, or, preferably, in the larger but usually well tolerated dose of 2 c c once weekly for twelve weeks. There is also available an ampule containing this larger dose, and another containing half the amount.

*d Tartro-Quimobine (N N R, 1932)*—Bulk dosage of this drug is perhaps more exact than that of the other suspensions listed above. The ampule commercially available contains 2 c c, one-half, or the entire, content of such an ampule should be injected twice a week for six to ten weeks. It is claimed that this preparation is superior in that its content of water soluble sodium potassium bismuthyl tartrate effects early action, and the insoluble bismuth iodide prolonged action, which has not yet been conclusively shown to be the case.

*e Bismuth Salicylate (N N R, 1932)*—One cubic centimeter of the commercial suspension is usually given once each week for ten weeks.

#### LIPOSOLUBLE PREPARATIONS

In the presence of certain lipid soluble substances, such as lecithin, matter may be satisfactorily dispersed in media in which it is otherwise insoluble. The first to make practical application of this fact to obtain optimal dispersion of bismuth were Heilmann and Nathan (1925),<sup>6</sup> whose studies have been continued by many other investigators.<sup>28</sup> One of the chief advantages urged in favor of these liposoluble preparations is that they become effective, through rapid absorption, almost as quickly as the water soluble salts, and that their absorption continues almost but not quite as long as that of the insoluble salts. These claims seem to be borne out by careful excretion studies. The injections are, furthermore, relatively painless and rarely lead to induration. Stomatitis probably occurs more often with these than with the water soluble preparations, but most of the cases are not severe. At the dedication of the new Dermatological Clinic in Strasbourg, in July, 1930, the subject of bismuth therapy in syphilis was discussed by many of the leading Continental syphilologists, and it was evident that the liposoluble preparations are gaining favor in Europe because of their dosage being more exact than that of the oily suspensions and because they do not cause the late abscesses of these latter, and also because the therapeutic results obtainable with them approach closely those obtained by the use of water soluble preparations. The greatest objection to their use is the necessity of injecting twice a week.

Perhaps the two best preparations available in this country, where experience with them has not yet been very extensive, are

*a Quimobine (N N R, 1932)*—Used in a dose of 1 to 2 c c twice weekly for twelve to fourteen injections.

*b Bismo-cymol*—Injected twice weekly, in doses of 2 c c, for twelve to sixteen injections.



## BISMUTH IN EARLY SYPHILIS

Levy (1930)\* divided a series of 66 cases into two parts, 11 he treated with various bismuth preparations, 25 with neoarsphenamine. With liposoluble preparations he caused the disappearance of spirochetes from the chancre as follows: in four days in 1 case, six days in 11 cases, 8 days in 1 case, and twelve days in 2 cases. With a soluble preparation the disappearance was accomplished in three days in 1 case, six days in 9 cases, and eight days in 1 case. With insoluble preparations the results were: six days in 1 case, seven days in 1 case, eight days in 1 case, eleven to twelve days in 8 cases, and sixteen to seventeen days in 3 cases. In the neoarsphenamine-treated cases the same result was accomplished in one day in eight cases with a dose of 0.3 gm, and in two days in 6 cases, 2 patients required a second injection of 0.45 gm. In 9 cases he used the very small dose of 0.15 gm neoarsphenamine and accomplished the desired result in one day in 3 cases, in the other six the disappearance was completed on the third day by a dose of 0.3 gm.

Of course the total number of Levy's cases is admittedly small and the two groups are not of comparable size so that statistically the study has no value, but I think that the results may nevertheless be fairly looked upon as fixing the status of bismuth very high in the treatment of primary syphilis but still below that of the arsphenamines. That the drug can quite satisfactorily replace mercury has been many times shown, perhaps no more conclusively than in Lomholt's<sup>3</sup> report of several years ago. He used neoarsphenamine in a series of five or six injections to the total number of about eighteen neoarsphenamine injections and sixty of bismuth in a period of about two years. In 152 patients thus handled there were no clinical relapses during or after treatment, no births of children with congenital syphilis, and only 2 positive, 7 doubtful, and 143 negative blood Wassermanns at the end of the treatment. Excellent results beyond doubt, but still there are those who maintain (Cole, in Cleveland, Moore, in Baltimore, O'Leary, at The Mayo Clinic, Parran, in New York, Stokes, in Philadelphia, Wile, in Ann Arbor),<sup>8</sup> and rightly, I think, that the great service of bismuth in early syphilis is as adjunct to or alternate drug with mercury, and not in displacing the older drug altogether. The well-known syphilologists listed just above alternate the three drugs through a course as follows: arsphenamine, bismuth, arsphenamine, mercury, arsphenamine, bismuth, arsphenamine, mercury, and still feel that final judgment on the relative merits of the two heavy metals is premature. That the profession is virtually abandoning mercury in favor of bismuth in early syphilis on the basis of individual results rather than long time statistical studies, seems to me most unfortunate.

A few men of great experience, particularly in France (Schwartz,<sup>9</sup> Emery, Rasis, Morin<sup>10</sup>), are using bismuth to the exclusion of all other drugs in all stages of syphilis, but they are certainly in the minority.

## BISMUTH IN LATE OR TERTIARY SYPHILIS

In this ubiquitous type of syphilis there can be no doubt of the great value of bismuth in rendering the use of the arsphenamines often unnecessary,

that is to say, since these cases can now be attacked by two metals, bismuth and mercury, as well as the iodides, it seems the part of wisdom to many syphilologists to employ the more dangerous and in many ways more objectionable arsenicals as little as possible. Since sterilization is very difficult here and holding the disease in check is the most to be hoped for, bismuth had best be used for all it is worth, taking care only, as Cole, Moore, O'Leary et al.<sup>8</sup> particularly stress, to keep the dose moderate in size. Quite remarkable results are sometimes obtained. McCafferty and MacGregor<sup>11</sup> gave 16 injections, then no treatment for a month then a blood Wassermann, in 25 patients who were asymptomatic with positive blood but negative spinal fluid, all of whom had had many courses of arsenic and mercury. Results after three full courses of the bismuth: percentage of patients improved as to Wassermann test, 64.7 (many rendered entirely negative from 4-plus), percentage unchanged, 22.9, percentage of reversions after second and third courses, 13.8.

#### BISMUTH IN NEUROSYPHILIS

All the preparations are used and with such excellent results at times that enthusiasm runs high in favor of displacing the arsphenamines entirely by the new drug. But the more conservative syphilologists here again favor merely adding the bismuth to the list of remedies, arsphenamines, triparasamide, mercury, iodides, hyperpyretic measures, which comprise the antineurosyphilitic armamentarium of today.

Iodobismutol may perhaps have some special virtues among the preparations, at least, Mehitens and Pouppirt (1931),<sup>12</sup> and Hanzlik, Mehitens et al (1932),<sup>2</sup> have shown that it penetrates well into the brain of experimental animals and that it also penetrates into the spinal fluid in the human being much better than control mercury preparations. About 50 per cent of their series of 100 patients of the several usual types of neurosyphilis were definitely improved clinically and serologically after one year's treatment, although many were in an advanced stage and proved intractable to other antisyphilitic medication. The earlier the treatment was initiated, the more satisfactory was the result.

#### BISMUTH IN CONGENITAL SYPHILIS

Unfortunately, bismuth is being too often tried to the exclusion of all other drugs, as was also the case when arsphenamine was first introduced. Still, there is some warrant for this practice in the case of bismuth, for when intravenous arsphenamine therapy is very difficult or impossible, and there are some other reasons for not using sulpharsphenamine intramuscularly, then there are only mercury and bismuth to fall back upon. And of the two, intramuscular injections of bismuth are very much better borne by infants and young children. An evaluation of the drug that is certain to stand long cannot be made yet because no considerable study appeared in the literature before 1925, and even now the reports are all upon small numbers of cases that have not been observed long enough to determine just what the therapy is going to accomplish ultimately. Coppolino's (1930)<sup>13</sup> report will serve to indicate methods and results. Dose  $\frac{1}{2}$  cc of bismuth salicylate to infants

under one year, 1 cc to older children. Course twenty weekly injections, with blood Wassermann at beginning and end of each course, if positive, treatment continued if negative, a rest period of four weeks allowed, the maximum number of courses was three. Results statistically the findings were of no importance, but the observation and impression was that the drug was most efficacious in young infants rendering them both symptom free and serologically negative and that it was useful but not curative in older children. The consensus of opinion is probably that bismuth used alone is a better drug than mercury alone but that it is not so good as the arsphenamines.

There are several reports of the use of bismuth after arsphenamine alone or with mercury had failed to reduce a 4-plus Wassermann in congenital cases. Wright,<sup>14</sup> for instance used the drug in 47 patients between two and sixteen years, averaging about ten years nearly all of whom had had considerable arsphenamine and mercury treatment and all but 4 of whom were strongly Wassermann positive. Dose 5 mg of potassium bismuth tartrate per kilogram, not to exceed 100 mg (the bismuth content of the weaker of the two NNR ampules) per week in courses of sixteen injections with a month's rest between courses. Result Wassermann reversal was obtained in 20 of the cases, or 47 per cent. Stokes<sup>15</sup> has reported a series in which arsphenamine and mercury injections, suitably carried out over a period of three to four years, accomplished reversal in 62 per cent of the cases, but of course it is notoriously inaccurate, and perhaps does not even have suggestive value, to compare two different series in this way.

#### BISMUTH IN ARSPHENAMINE-INTOLERANT CASES

Here of course the drug has tremendous value because these patients have a far better chance of having the disease arrested in them with bismuth and mercury than they formerly had with mercury alone.

#### THE TOXICITY OF BISMUTH

One of the greatest advantages of this drug over both mercury and the arsphenamines is that, though the reactions of a toxic nature which accompany its use are many and varied, they are rarely severe enough to necessitate an interruption of the treatment for longer than a few days. The commonest of the symptoms is the appearance of a thin violaceous gray line on the gums, indicative of saturation. Experience has taught that it is well to examine the urine of patients before beginning the treatment and to use the findings as a quantitative guide to the same. Now and then during the courses the urine even of patients having no preexisting nephritic disturbances will give evidence of a beginning toxic process in the kidneys, but this does not happen as often as with mercury perhaps and certainly only rarely fails to disappear with adjustment in dosage or after a slight pause in treatment. According to Heimann-Trosien (1928),<sup>16</sup> in most instances the kidneys acquire an increased tolerance for the metal, but the fact is not established and is probably open to considerable doubt (Taralrud, 1931).<sup>17</sup> Very rarely indeed has a severe or long-lasting nephritis been reported.

The single other symptom of a deleterious nature which is relatively often noted (but not nearly so often as with mercury) is stomatitis, it is usually of a mild sort and disappears promptly upon adjustment of the treatment schedule or dosage. Of the local reactions upon injection I have already treated above. Other rare occurrences: loss of ambition, asthenia, etc (bismuth "grippe"), herpes zoster, purpura hemorrhagica, agranulocytosis, nitritoid crises, polyneuritis, various exanthemas and dermatoses, diarrhea, jaundice, menstrual disturbances, etc. With the more serious reactions of bismuth introduced intravenously we need not concern ourselves, since the introduction of the drug by this route is absolutely contraindicated.

#### SUMMARY

1 The water soluble bismuth salts are rapidly and fairly regularly absorbed but usually cause pain on injection and must be injected too often to be ideally suited for use in either private or clinic practice.

2 The only suspensions are more irregularly and slowly absorbed than the aqueous solutions and may cause embolism or infarction or be followed by troublesome abscesses, but they need be injected only once a week and the injections are not usually very painful. Only the use of individual ampule preparations insures reliable dosage.

3 The absorption of the liposoluble preparations begins almost as quickly as that of the aqueous solutions but proceeds more nearly at the slower rate of the oily suspensions. The injections are relatively painless and rarely leave sequellae, they must, however, be made as frequently as when using aqueous solutions.

4 In early syphilis bismuth can apparently quite satisfactorily replace mercury but should not be exclusively so employed until long-time statistical studies have proved its superiority beyond doubt.

5 In late syphilis there seems to be no doubt that bismuth, properly used with mercury and the iodides, often makes the use of the arsphenamines unnecessary, Wassermann reversal is sometimes accomplished in very stubborn latent cases.

6 In neurosyphilis bismuth has proved its right to be added to the list of effective agents but not to usurp the place of any of the others.

7 In congenital syphilis when used alone bismuth is of greater value than mercury alone but probably less valuable than the arsphenamines. It sometimes accomplishes Wassermann reversal where the other two drugs have failed.

8 In arsenic-intolerant cases bismuth is of great value as an alternate drug with mercury.

9 The toxicity of bismuth is much lower than that of either the arsphenamines or mercury.

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## THE EFFECT OF NUTRITIONAL STATUS ON THE PHOSPHORUS CONTENT OF PROTEIN OF TISSUE\*

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I DESIRED to determine any alteration in phosphorus content that might occur in the proteins of muscle and liver during changes in states of nutrition of animals, or during the development of metabolic diseases, with the hope that this might contribute information on the "reserve" protein. Tissues from animals representing different stages of nutrition were first employed, but because of the possibility of individual variations in phosphorus content of protein, tissues were obtained from the same animal at different stages of nutrition, or before and after the development of a disease. The tissues were obtained by an operative procedure. Dogs and swine were studied, also the livers of four human subjects were analyzed, three represented different stages of nutrition and one was myxedematous.

This work is submitted to show that the phosphorus content of the protein of muscle and liver of dogs, and of the liver of human subjects, was greatest in the emaciated subject that no changes of the phosphorus content of protein occurred in the swine during the development of cretinism, and that the content of the human liver was greater than that of the swine or dog.

### LITERATURE

Kossel found that the phosphorus content of protein of the livers of lean fowls was greater than that of obese fowls. Kiuger found that the phosphorus content of the livers of man and bees was greater during the pre-natal period. Grund concluded that the percentage of total phosphorus was greater in the livers of emaciated dogs and fowls. King stated that the phosphorus content of protein of dog's muscle was increased during fasting. Francis and Trowbridge thought that the breed and prefattening diet might have something to do with the content of phosphorus found in the tissues of animals. They found that in raw beef 75 per cent of the total phosphorus was in organic form, that fats contained little phosphorus, that the flesh of thin animals contained more water-soluble phosphorus than that of obese animals, that the percentage decreased with increasing obesity, even when considered on a moisture and fat-free basis, and that age was not a factor. Greenwald found a diminution of phosphorus output after parathyroidectomy. Sherman found that the phosphorus requirement for man of about 70 kg appeared to be about 0.88 gm a day. Bruce stated that in the herring the phosphorus content of the isolated protein of the liver was higher than that of the muscle.

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†Fellow in Surgery. The Mayo Foundation

Cahn and Bonat demonstrated that the isolated protein of the livers of emaciated dogs, guinea pigs, and rabbits contained a higher percentage of phosphorus than did the same protein of the respective obese animals.

#### METHOD OF PROCEDURE

All animals were kept in cages which were provided for separate collection of feces and urine, and they had free access to distilled water. Other observations and analyses were made of the animals and will be reported in another paper. Partial hepatectomy, which consisted of the removal of the left lateral lobe, was performed according to the method of Mann and Graham, and at the same time the right adductor longus muscle was excised and used for analysis, at death, the middle lobe of the liver and the left adductor longus muscles were used. All animals were operated on under ether anesthesia and all were killed by letting blood under ether anesthesia.

Dog 1 was a well-nourished female collie weighing 12 kg. Dog 2 was a female mongrel that had been fasted thirty days, during which time it had lost 3.8 kg in weight. Dog 3 was a female collie that was observed for eighty-one days. Its weight was gradually reduced from 13.6 kg to 10.2 kg where it remained stationary during the last twenty-six days when the dog was on a maintenance diet. Dog 4 was a very obese female collie weighing 16.4 kg at the time of operation. It was fasted forty-four days and weighed 9.4 kg at the time of death. Dog 5 was a female collie that weighed 10.6 kg at the time of operation. After recovery from the operation, the dog was fed 400 gm daily for the first twenty days, and 600 gm daily for the last eleven days, of a mixture containing 44 per cent lean horse meat, 44 per cent cracker meal, 8 per cent lard, and 4 per cent bone ash. The dog attained a weight of 14.4 kg at the time of death. Dog 6 was an underweight mongrel that weighed 8.8 kg when operated on, after recovery it was fed lean horse meat for six days until nitrogen equilibrium was established, then it was fasted fourteen days and killed, it weighed 6 kg.

The swine were given shelled corn and water. Swine 1 weighed 30 pounds at the time of thyroidectomy. It died forty-two days later after the development of cretinism. Swine 2 weighed 75 pounds at the time of total thyroidectomy, removal of a wedge-shaped piece of the liver, and excision of the right adductor longus muscle. It was killed sixty-two days later, after cretinism had developed.

Livers from four human subjects which had been preserved in the Robertson-Lundquist modification of Kaiserling's solution were analyzed.

The liver was pressed between absorbent cloths to free it of blood. It was then forced through screen wire (sixty meshes to the square inch<sup>13</sup>) thus separating the parenchyma, which was reduced to the consistence of thick buttermilk, from the stroma. The fixed human liver was reduced to small particles by a nutmeg grater, the stroma remained behind as long shreds. The muscle was freed of fat and fascia and was macerated by the mortar apparatus. The protein of all tissue was isolated by using Green's modification of Janney's method. The dried isolated protein was analyzed for phosphorus according to the method described.<sup>5</sup>

## RESULTS

The data in Table I suggested that the isolated proteins of muscle and liver of emaciated dogs contained a higher content of phosphorus. The possibility of individual variations was eliminated in Dogs 4 and 5 when the same animal in one case lost weight with an increase in the percentages of phosphorus in the isolated proteins of muscle and liver, whereas the converse occurred in the animal that gained weight. Dog 6 showed no change in the protein phosphorus considered despite a loss in weight. It was emaciated at the beginning of the experiment and probably had to live on its own bioplasm as the "reserve" protein had already been exhausted, an idea further supported by the comparatively high percentage of phosphorus content of the proteins at the beginning of the study.

TABLE I  
DOGS STUDIED

DOG	BREED	INITIAL WEIGHT, KG	FINAL WEIGHT, KG	CHANGE OF WEIGHT, PER CENT	DAYS FED	DAYS FASTED	COM- MENT	PRO- TEIN	PHOS- PHO- RUS,* PER CENT	CHANGE OF PHOS- PHORUS PER CENT
1	Collie	12.0	12.0	0			Very obese	Muscle Liver	0.099 0.206	
2	Short hair	12.0	8.2	-31.66			Under weight	Muscle Liver	0.106 0.326	
3	Collie	13.6	10.4	-23.52			Under weight	Muscle Liver	0.123 0.320	
4†	Collie	16.4						Muscle Liver	0.094 0.217	
			9.4	-42.7		44		Muscle Liver	0.117 0.437	+23.9 +83.6
5†	Collie	10.6						Muscle Liver	0.091 0.316	
			14.4	+35.8	31			Muscle Liver	0.085 0.268	- 6.6 -13.6
6‡	Short hair	8.8						Muscle Liver	0.120 0.312	
			6.0	-31.8	6	15		Muscle Liver	0.119 0.310	0.0 0.0

\*Phosphorus content of isolated proteins was greater in the underweight dogs.

†Phosphorus content of protein of isolated muscle and liver increased 23.9 per cent and 83.6 per cent respectively when the dog had lost 42.7 per cent in weight.

‡Phosphorus content of protein of isolated muscle and liver decreased 6.6 per cent and 13.6 per cent respectively when the dog had gained 35.9 per cent in weight.

§Phosphorus content of protein of isolated muscle and liver remained constant despite loss of weight.

TABLE II  
SWINE STUDIED

SWINE	INITIAL WEIGHT, LB	INTERVAL, DAYS	FINAL WEIGHT, LB	COMMENT	PROTEIN	PHOSPHORUS, PER CENT
1*	30	47 (died)	30	Cretin	Muscle Liver	0.134 0.370
2†	75	62 (moribund)	68	Cretin	Muscle Liver	0.100 0.330
					Muscle Liver	0.100 0.325

\*Died forty-seven days after total thyroidectomy. Control swine prospered on same diet.

†Development of cretinism caused no change in phosphorus content of isolated protein. Swine was moribund when killed.



TABLE III  
PATIENTS STUDIED

PATIENTS	BODY NUTRITION	WEIGHT OF SUBJECT, LB	CAUSE OF DEATH	PHOSPHORUS,* PER CENT
1	Poorly nourished	140	Ureteral calculus	0.461
2	Well nourished	165	Hemorrhage	0.440
3	Obese, graded 2	180	Skull injury	0.395
4	Obese, graded 4	200	Myxedema	0.147

\*Phosphorus content of isolated protein of liver was greatest in most emaciated subjects in a myxedematous subject it was intermediate

Swine 1 and 2 (Table II) did not live on shelled corn and water after total thyroidectomy, although normal swine prospered for a period of sixty days. Calcium in the blood was normal in each case and necropsy revealed evidences of metabolic disturbance only.

The protein phosphorus content of livers of human subjects (Table III) varied also according to the state of nutrition, with the exception of that of the myxedematous subject which was intermediate. The fact that myxedema is characterized by altered protein metabolism probably accounted for this result.

## SUMMARY

Phosphorus changes occurred in the isolated proteins regardless of whether the state of nutrition had been produced acutely, as in the case of Dog 4, or had existed chronically, as in human subjects. The phosphorus content of protein was greater in the more emaciated subjects with but one exception which might probably be accounted for by the reserve protein, as suggested by Cahn and Bonat.

No changes in the content of phosphorus in the protein occurred in the swine during the development of cretinism. In case of human beings the livers of the more emaciated subjects contained more phosphorus in the protein with the exception of the one that was myxedematous.

The changes as well as the percentages of protein phosphorus in the liver were greater than those of muscle at different levels of nutrition.

The variability in the phosphorus contents of different isolated proteins may account partly for the varying results given by different authors.

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## INVESTIGATIONS CONCERNING THE EFFECT OF BLOOD TRANSFUSION ON THE HEART\*

### I ELECTROCARDIOGRAPHIC STUDIES

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**I**T IS not always safe to perform a blood transfusion on an individual known to be suffering from cardiac or cardiovascular disease. In the performance of about 3500 transfusions the writer (P) has noticed a number of reactions which seemed to be due to cardiac embarrassment, all other known causes having been excluded. Three illustrative cases of this type are cited briefly.

CASE 1—L G, aged sixty seven, a case of carcinoma of the pancreas with severe jaundice developed bleeding from a cholecystgastrostomy wound. A blood transfusion was ordered to replace the loss of blood and to improve her general condition. After having received 450 cc of compatible blood, the patient became very faint and feeble and requested that the transfusion be stopped. Her request was granted but the patient lost consciousness, became cyanotic, developed clonic convulsions and died within two minutes after the onset of symptoms. There was no postmortem examination.

CASE 2—H U, aged twelve, a case of active rheumatic endocarditis presented signs of decompensation and marked anemia. A blood transfusion was requested by the attending physician to combat the anemia. The child had received 200 cc of blood when she began to complain of numbness in the arm receiving the blood, became faint, and developed marked cyanosis, an imperceptible pulse, and stertorous breathing. The transfusion was stopped at once but the patient lost consciousness and showed signs suggestive of a cerebral accident. She died within five minutes after the onset of the symptoms. A postmortem examination was not permitted.

CASE 3—A woman, fifty years of age was in a condition bordering on shock following the removal of huge fibroids of the uterus. Her cardiac condition prior to operation was poor. A blood transfusion was ordered to improve her general cardiovascular tone. After having received 400 cc of blood, the patient developed symptoms similar to those described in Case 2, but the progress of the symptoms ceased with the cessation of the transfusion. The patient eventually recovered but remained very weak for several days.

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In no case of this group could the reaction be ascribed to incompatible blood or other demonstrable cause. Since there was evidence of existing cardiac pathology in these cases various experiments were undertaken to study the effects of transfusion, in order to determine if possible, the rôle played by the heart in these reactions. The present report deals with *electrocardiographic* observations made before, during and after blood transfusions performed on patients with normal and diseased hearts. Since no records of similar studies on the human being could be found in the available literature, the results of this investigation were considered to be of sufficient value for publication.

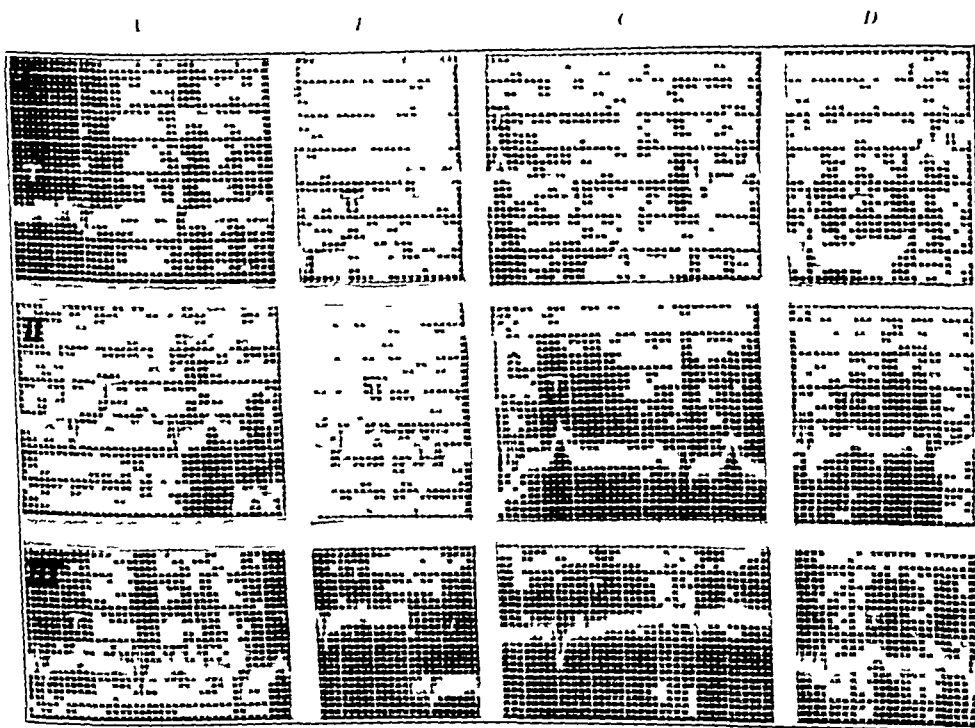


Fig 1—(A) Tracing previous to removal of blood (B) Removal of 100 cc of blood (C) Removal of 200 cc of blood (D) Addition of 500 cc of blood. Note the progressive increase in the height of the T-waves in Leads I and II and a decrease in height of T-waves in Lead III upon removal of blood. Also note return to original form of T-waves in all leads upon addition of blood.

The cases were divided into four groups, according to the cardiac condition of the patient as determined clinically and by the electrocardiograph before transfusion. Group I consisted of patients with normal hearts, Group II those with chronic valvular diseases, Group III, those with myocardial degeneration and coronary diseases, and Group IV, those with acute and subacute endocarditis.

In each instance an electrocardiographic tracing was first obtained, after which the patient was prepared for the transfusion. No difficulties were encountered in draping the patient or keeping the field sterile for the transfusion. As the latter proceeded, the electrocardiographic studies were resumed



TABLE I—Contin'd

CIST NO	BLOOD GROUP	AGE	DIAGNOSIS	CARDIAC CONDITION	VOLUME GIVEN	PAC CHANGES DURING OR AFTER TRANSFUSION	TRANSFUSION RATE			TRANSFUSION REACTION
							BEFORE	DURING	AFTER	
1	A	69	Carcinoma of stomach	Coronary sclerosis	500 cc	None	53	83	79	None
2	A	72	Arteriosclerosis Myocardial insufficiency	Coronary sclerosis Myocardial insufficiency	300 cc	None	53	94	91	None
3	O	32	Chronic nephritis Uremia	Coronary sclerosis Myocardial insufficiency	250 cc	Tendency toward right axis deviation (temporary)	111	177	113	None
4	O	62	Hypertension in uremia Second	Ventricular extrasystoles Coronary sclerosis Myocardial damage	300 cc	Auricular extrasystoles (temporary)	86	81	75	None
5	A	61	Leucemic lymphadenosis	Myocardial damage	500 cc	None	103	105	94	None
6	O	70	Duodenal ulcer Arteriosclerosis	Myocardial damage	300 cc	None	79	75	75	None
7	O	18	Hemorrhage Fibrinolytic telangiectasis	Myocardial damage Auricular fibrillation	500 cc	None	100	100	100	None
8	O	60	Anemia	Myocardial damage	400 cc	None	115	125	125	None
9	O	56	Chronic nephritis Uremia	Coronary occlusion	350 cc	None	111	103	100	None
10	B	73	Ossler's disease	Myocardial damage	300 cc	Tendency toward right axis deviation	103	115	111	None
11	A	25	Glomerulonephritis Uremia	Myocardial damage	500 cc	Change in T wave*	103	79	75	None
1	B	22	Rheumatic polyarthritis	Mitral and aortic stenosis Myocardial damage	300 cc	None	86	81	81	None
2	O	12	Acute rheumatic fever Bronchopneumonia	Myocardial damage	200 cc	None	130	136	125	Post transfusion chill Hyperpyrexia None
3	A	31	Subacute bacterial endocarditis	Myocardial damage	300 cc	None				
4	O	36	Subacute bacterial endocarditis	Myocardial damage	500 cc	None	136	136	142	1 cft f unit during transfusion

\*On removal of 200 cc of blood a change in T-wave was noted which tended to return to previous condition on receiving 500 cc of blood (see Table 1)

GROUP III

GROUP IV

and the number of cubic centimeters of blood injected was noted at regular intervals during the tracings. A final electrocardiographic record was made soon after the completion of the transfusion. The data collected in this study have been tabulated in Table I.

A study of the table fails to reveal any *significant* electrocardiographic changes which could be ascribed to the effects of blood transfusion in any of the cases studied.

Those of Group I (normal hearts) failed to show any changes, either during or after the blood transfusion.

In Group II (chronic cardiovalvular diseases), one case (No. 1) showed a change toward right axis deviation. This change, however, may have been caused by a shift in the position of the patient, a circumstance which frequently cannot be avoided during the preparation of the patient for transfusion. It is also noteworthy that in Case 3 the patient complained of marked precordial oppression, yet the tracing failed to reveal any changes.

In Group III (coronary sclerosis and myocardial degeneration), there was a change toward right axis deviation in two instances. More interesting observations, however, were made in two other cases of this group. Case 4, which showed ventricular extrasystoles in the pretransfusion tracing during the transfusion showed the presence of auricular extrasystoles which disappeared after the completion of the transfusion. Since a repeated study of this case was not possible, an explanation of this observation cannot be offered. In Case 11 200 cc of blood was first removed during which time an electrocardiographic tracing was made. This tracing showed a change in the T-wave which tended to return to the previous condition on replacement of 500 cc of blood (see Fig. 1). Apparently the removal of blood caused this change. Further studies are now being made from electrocardiographic tracings on donors during transfusions to confirm this observation.

The cases of Group IV (acute and subacute endocarditis) showed no electrocardiographic changes whatever.

#### CONCLUSIONS

Electrocardiographic studies throw little light on the changes in the heart during blood transfusion, even though there is definite clinical as well as electrocardiographic evidence of existing heart disease. In one case there were definite anginal symptoms during the transfusion with no changes in the electrocardiographic tracing. Other means than the electrocardiograph must be sought as an aid to determine the effects of transfusion on a diseased cardiovascular system.

# THE LARGE Q WAVE IN LEAD III OF THE ELECTROCARDIOGRAM\*

## A COMPARISON OF THE WHITE AND NEGRO RACES

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IN VIEW of the greater incidence of coronary thrombosis in the white race than in the negro, and of the recent interest in large Q waves in Lead III of the electrocardiogram as evidence of coronary occlusion, we thought it would be worth while to compare the incidence of the Q wave in the two races. Accordingly every example of the large Q wave was removed from our files and the whole group, 212 in all, was studied. In making our selection the criteria given by Pardee were adhered to. Only those Q waves were chosen whose amplitude amounted to 25 per cent or more of the amplitude of the highest R wave of the three leads, and the electrocardiograms of right axis deviation with deep S in Lead I were excluded. In every case, also, an upward excursion, R, followed the Q.

The frequent occurrence of the large  $Q_3$  in coronary thrombosis was first noted by Pardee (1924),<sup>1</sup> and was studied in more detail by Levine (1930).<sup>2</sup> More recently papers dealing with this sign have been written by Pardee,<sup>3</sup> by Willius,<sup>4</sup> by Fenichel and Kugell,<sup>5</sup> and by Edeiken and Wolferth.<sup>7</sup> Pardee and Willius were primarily interested in the incidence of cardiac pain and arteriosclerotic heart disease among the group of patients with the Q wave, while Fenichel and Kugell presented evidence from pathologic studies that the Q wave is associated with disease of the interventricular septum, particularly the lower and posterior portion. In Pardee's series of  $Q_3$  patients, 63 per cent presented the anginal syndrome, in Willius' series, 38.3 per cent. It may be stated at this point, that the incidence of cardiac pain in our series, in white patients, falls between these two figures, namely, 47 per cent, or if we exclude from our calculation the small number in whom our records are questionable regarding pain, 54 per cent.

*The Incidence of Large  $Q_3$  Among Whites and Negroes*—The electrocardiograms in our files which fulfilled the criteria given by Pardee were studied, in all 212 from 5960 patients, of whom approximately 4870 had heart disease. Of the 5960 patients, 3810 were white and 2150, colored, 64 per cent and 36 per cent respectively. In the white group approximately 2905, or 76.3 per cent had heart disease, in the negro group 1965, or 91.5 per cent.

\*From the Heart Station of Charity Hospital and the Departments of Physiology and Medicine of the Medical Center Louisiana State University.

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†These were taken during a period of about six years the majority while Dr George Hermann was director of the Heart Station.

Of the 212 large Q waves in Lead III, 160 were obtained from white and 52 from colored patients. The incidence among the white and colored groups is therefore, 4.2 and 2.4 per cent, respectively. If, however, we exclude all examples of large Q wave found in patients in whom no diagnosis of cardiac disease could be made, and also exclude this group from the total number of patients, the incidence in the white and negro groups becomes 4.9 and 2.5 per cent respectively. The reason for this change is that many more white than colored patients entered the Heart Station without frank evidence of heart disease. It can be stated, therefore, that the incidence of large Q waves in Lead III of the electrocardiogram is twice as great among white as among colored patients with cardiac disease. Looking at the same fact in a different way, although well over a third, 36 per cent, of all patients were negroes, they contribute but 2.45 per cent, i.e., less than a fourth, of all the large Q waves. This difference would be more striking if all cases without definite heart disease were excluded.

The reason for this racial difference is probably to be found in the greater incidence of coronary artery disease in the white race, a difference which cannot be wholly explained on the assumption that negroes, in general, do not live long enough to develop coronary sclerosis. This is indicated by the following. The average age of all white patients admitted to the Heart Station was about 42 years, 57 per cent of whom were 40 or above, and 41 per cent, 50 years or over. The average age of the negro patients was 41 years, 57 per cent of whom were 40 or over, and 32.5 per cent, 50 or above. In contrast to this small difference, the average age of the white patients in the large Q<sub>3</sub> group was 54, of the negroes 41.5. We interpret this to mean that those factors, other than coronary disease, which tend to produce a large Q wave may operate at an earlier age, while coronary disease, which is responsible for the majority of the large Q waves in whites, is found most frequently at ages beyond 50. Factors other than coronary disease which tend to produce large Q waves were in the minority among the white patients, but in the majority among the negroes.

*The Etiologic Factors Responsible for the Large Q Wave*—Even without resorting to more involved statistics, it is apparent that coronary thrombosis, and the analogous pathologic changes which occur in certain cases of angina pectoris are the most important single cause in the production of the large Q wave in Lead III. Among the 120 white males with the Q wave, no fewer than 24 presented a typical clinical picture of coronary thrombosis, but only 1 among the 40 white females. In contrast with this, only 2 of the 52 negroes in this group, both of whom were females, gave a history even slightly suggestive of coronary thrombosis. The incidence of the various etiologic factors in the production of heart disease in the white and negro patients seen in the Heart Station is given in Table I. It is, of course, recognized that no definite separation can be made of arteriosclerotic and hypertensive heart disease. All patients with systolic and diastolic hypertension are here classed as hypertensive. The arteriosclerotic group includes those without diastolic hypertension. The average ages of our hypertensive patients were as follows: white males 56.5, white females 51, colored males 51, colored females 47. In



view of this racial difference in the age of development of cardiac symptoms in hypertension (and probably in onset of the condition), it is probable that many, if not most, of the hypertensive white males had also some degree of coronary sclerosis while in the colored females the reverse is probably true

TABLE I

ETIOLOGY	HEART STATION POPULATION		LARGE Q <sub>3</sub>		CONTROL GROUP	
	WHITE	COLORED	WHITE	COLORED	WHITE	COLORED
Arteriosclerotic	26.9	13.1	46.2	13.5	39.1	11.5
Hypertensive	13.6	23.6	25.0	28.8	19.1	25.0
Syphilitic	8.7	31.1*	6.9	28.8	8.8	32.7
Rheumatic	12.7	9.8	1.1	7.7	6.9	9.6
Hyperthyroid	2.1	2.3	0.6	1.9	1.2	3.8
Other, Including Unknown	11.9	8.5	7.5	13.5	7.5	5.8
No Definite Heart Disease	23.8	8.6	10.6	5.8	16.9	11.5

\*We are indebted to Dr. Herrmann for the criticism that the criteria upon which a diagnosis of syphilitic aortitis was based were often inadequate particularly in the negro. In a considerable percentage of this group in spite of a positive Wassermann reaction or history of syphilitic infection together with other suggestive signs the true diagnosis was probably arteriosclerotic heart disease rather than syphilitic. This group does not include hypertension however even when there was also a strong presumption of associated syphilitic vascular disease.

For comparison with these data the incidence of the various etiologic factors among white and colored patients whose electrocardiograms presented large Q waves in Lead III is also shown in the table. A comparison of the first and second parts of the table brings out the fact that arteriosclerotic-hypertensive heart disease although constituting 41.5 per cent of all white admissions to the Heart Station, supplied 71.2 per cent of all the large Q waves in the whites, while among negroes the corresponding figures are 37.5 per cent and 42.3 per cent. In white patients, syphilitic heart disease contributes less than its quota of Q waves, and the same is true to a more striking degree of rheumatic heart disease and other conditions, including those in whom a single examination revealed no definite evidence of heart disease (*vide infra*, Q<sub>3</sub> with no heart disease). In negro patients the percentages are less reliable because of the relatively small number of cases. It appears, however, that the arteriosclerotic-hypertensive group contributes slightly more and the syphilitic group somewhat less than their due proportions of Q waves.

In view of the fact that the average age of the patients with large Q waves is greater than that of patients as a whole, we felt that the control figures we have presented fall short of proving that there is any necessary connection between arteriosclerotic heart disease and incidence of the Q wave, since another group of the same age, but without the Q wave, might show the same incidence of that etiologic factor. Accordingly we selected 212 cards at random, matching every patient in the Q wave group with another patient of the same age (usually of identical age, but always within a year or two), sex and color, but without the Q wave. This control series, therefore, is of the same sex, color, and average age as the Q wave group. In the last line of the table we give the etiologic factors involved. It will be noted that the arteriosclerotic-hypertensive group, 71.2 per cent in the Q group, falls to 58.7 per cent in the white control group, while every other etiologic

factor in the control rises in percentage, notably, the diagnosis of no demonstrable heart disease. The increase in the syphilitic group is not great. By such a comparison we again demonstrate the importance of arteriosclerotic heart disease as the chief factor in the production of the large Q wave in Lead III.

*Incidence in the Large Q<sub>3</sub> Group of Otherwise Normal Electrocardiograms*—In Willhus' 300 electrocardiograms exhibiting the large Q wave in Lead III, the Q wave was regarded by him as the only significant electrocardiographic feature in 198 cases (66 per cent). He says: "Therefore, the abnormality may be looked on as being an additional diagnostic sign that may be of reliable significance when the electrocardiogram is otherwise unaltered."<sup>4</sup> We agree with this statement and also with the conclusion of Fenichel and Kugell,<sup>5</sup> namely, that often, after the infarcted myocardium has healed the Q may be the only evidence of past thrombosis. However, in our series of 212 electrocardiograms with the Q wave we found definite electrocardiographic evidence of myocardial disease in the majority. Of the 160 electrocardiograms of white patients, 34.3 per cent were normal, except for the presence of the Q, of the negro patients, 23.1 per cent.

*The Incidence of Cardiac Pain* among patients whose electrocardiograms reveal the large Q wave also serves to emphasize the contrast between the races, and in the white race, between the sexes. The incidence of pain was as follows:

White males: pain 64 or 53.3 per cent, no pain 40 or 33.3 per cent, doubtful 15 or 12.5 per cent.

White females: pain 11 or 27.5 per cent, no pain 22 or 55 per cent, doubtful 7 or 17.5 per cent.

Colored males: pain 4 or 19 per cent, no pain 16 or 76 per cent, doubtful 1 or 5 per cent.

Colored females: pain 9 or 29 per cent, no pain 14 or 45 per cent, doubtful 8 or 26 per cent.

The incidence of pain among the white males, therefore, was much greater than in any other group. White females show more pain than the negro group as a whole. The difference in pain between negro males and females is probably not significant, unless it, and the excess of large Q waves among the colored females, reflects the greater incidence among them of hypertensive heart disease. That the colored female exhibits a higher proportion of hypertensive heart disease than the colored male is shown by Schwab and Schulze,<sup>6</sup> a finding we can confirm.

Our records show very conclusively that the incidence of cardiac pain among white patients as a whole (19.8 per cent) is much less than among this Q wave group. But obviously a true comparison must be with another group of the same average age, sex, and color. The following figures are from the control group of 212 patients already mentioned:

White males: pain 36 or 30 per cent, no pain 68 or 57 per cent, doubtful 16 cases or 13 per cent.

White females: pain 8 or 20 per cent, no pain 22 or 55 per cent, doubtful 10 cases or 25 per cent.

Colored males pain 5 or 24 per cent, no pain 13 or 62 per cent, doubtful 3 or 14 per cent

Colored females pain 9 or 29 per cent, no pain 17 or 55 per cent, doubtful 5 or 16 per cent

The remarkable facts brought out by this are the significantly smaller incidence of cardiac pain among the white males in this than in the Q wave group and the absence of any significant difference for the other sex and color classes. Probably a sufficiently large series of cases would bring out a slight difference, at least among the white females.

*The Incidence of Large Q<sub>3</sub> Among Patients With No Evidence of Heart Disease*—For this study 150 electrocardiograms were chosen of persons whose electrocardiograms were taken as normal controls or of patients whose hearts were definitely regarded as normal. Excluded from this group are those who are entered in our records as cases with "no definite heart disease." Many of this latter classification would now be diagnosed "possible heart disease," or "potential heart disease." Of this group of 150 normals, only 3, or 2 per cent, presented the large Q wave. Of these 3, however, two were not typical and were barely included on the basis of the criteria given by Pardee. These Q waves, although deep, were narrow and Lead I presented a well-marked S wave. We are inclined to agree with other authors that wide, well-marked Q waves in Lead III, are nearly always evidence of myocardial disease.

*The Incidence of Q<sub>3</sub> Among Patients With Cardiac Pain*—Among 101 white males with cardiac pain, the incidence of the large Q wave was 16 per cent, among 61 white females, 10 per cent, among 48 colored males, 6 per cent, and among 41 colored females, 2.5 per cent. These figures are sufficient to prove that the incidence of the large Q wave is much greater among white patients with cardiac pain than among cardiac patients in general.

*The Incidence of Q<sub>3</sub> Among Patients With a Definite Clinical Diagnosis of Coronary Thrombosis*—Sixty clear cases were selected at random. Of these 23, or 38 per cent, exhibited the large Q wave in Lead III.

*Is the Significance of the Large Q Wave Identical With That of Other Evidences of Defective Intraventricular Conduction?* The work of Fenichel and Kugell brought out most clearly that the large Q wave in Lead III is a consequence of damage of the interventricular septum, and as such, is evidence of defective intraventricular conduction. May it not be true, therefore, that any picture of defective intraventricular conduction, such as is indicated by marked slurring and notching of the QRS group, is equally good evidence of coronary thrombosis? In order to test this point we chose 112 electrocardiograms of white patients and 95 electrocardiograms of colored patients which exhibited conspicuous slurring and notching or else the classical picture of bundle-branch block. Of the 112 white patients, 23 per cent gave a definite history of pain, 20 per cent were classed as doubtful, while 57 per cent had had no pain. The corresponding percentages in the negro group were 28, 17, and 55 per cent, respectively. There is no significant difference. We may conclude, therefore, that the Q wave is of rather special significance. It is, much more than slurring, notching or bundle-branch block, likely to mean coronary thrombosis or angina pectoris. Furthermore, the above figures for

white and colored, 112 and 95, are proportional to the incidence of defective intraventricular conduction in the two races. The negro (with due allowance for the inclusion of more normals and children among the electrocardiograms of our white patients) has even more than his due proportion of this type of abnormal electrocardiogram. This is in contrast to the situation with respect to the Q wave. Syphilitic and hypertensive heart disease are the prime producers of defective intraventricular conduction in the colored, arteriosclerotic and hypertensive in the white race in New Orleans.

#### SUMMARY

1 In the electrocardiograms taken on 5960 patients admitted to the Heart Station of the Charity Hospital, the incidence of a large Q wave in Lead III was twice as great among white as among negro patients with heart disease.

2 Although the average ages of white and colored patients admitted to the Heart Station were approximately the same (42 and 41 years, respectively), the average age of the white patients in the  $Q_3$  group was significantly greater than that of the negro patients (54 and 41.5 years, respectively).

3 Arteriosclerotic-hypertensive heart disease constituted 41.5 per cent of all white admissions to the Heart Station, but supplied 72 per cent of all the large Q waves in this race. Other etiologic types of heart disease contributed less than their due quota of Q waves in white patients. Among negro patients arteriosclerotic-hypertensive heart disease supplied but slightly more than its due quota of large Q waves.

4 In a control group of records, each patient in the  $Q_3$  group being matched with another of the same age, sex, and race, but whose electrocardiogram did not present a large  $Q_3$ , the incidence of arteriosclerotic-hypertensive heart disease was significantly less among the white patients (58.7 per cent) and slightly less among the negro patients than in the  $Q_3$  group.

5 The incidence of cardiac pain was significantly greater among white males in the  $Q_3$  group than in the control group, but this difference did not obtain in the other sex and color classes.

6 Among 150 electrocardiograms taken on persons whose hearts were regarded as normal only one showed a typical wide well-marked Q wave in Lead III.

7 In our records the incidence of a large  $Q_3$  was much greater in white patients with cardiac pain than among patients with heart disease in general.

8 Of 60 cases of coronary thrombosis 38 per cent exhibited the large  $Q_3$ .

9 Among a group of patients whose electrocardiograms showed well-marked evidence of defective intraventricular conduction but no  $Q_3$ , the incidence of cardiac pain in the white race was only half as great as in the  $Q_3$  group, but in the negro race there was no significant difference in the two groups.

10 Of the electrocardiograms exhibiting the large  $Q_3$ , approximately a third of those from white patients and a fourth of those from negro patients were otherwise normal.

## CONCLUSIONS

1 Arteriosclerotic heart disease is the chief but not the only factor in the production of large Q waves in Lead III of the electrocardiogram

2 The much greater incidence of large Q waves in Lead III in the white than in the negro race is probably due to the greater prevalence of arteriosclerotic (coronary) disease in the white race

3 Other factors than coronary disease, which may operate at an earlier age, produce a large portion of the large Q waves in negroes, while coronary disease is responsible for the majority of large Q waves in the white race

4 In white patients, especially white males, a large  $Q_3$ , much more than other electrocardiographic evidence of defective intraventricular conduction, is likely to signify angina pectoris or coronary thrombosis

5 We agree with other authors that wide, well-marked Q waves in Lead III are nearly always evidence of myocardial disease

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## COMPARATIVE VALUE OF THE INTRADERMAL AND OPHTHALMIC TESTS FOR SENSITIVENESS TO HORSE SERUM\*†

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THE frequency and severity of serum reactions have increased considerably during the past decade. This is attributable partly to the more widespread employment of immune sera, but more likely to the necessity for the administration of such serum into individuals sensitized to horse serum by previous injection of either immune sera or, in the case of children, toxin-antitoxin mixtures. In 1929 Gordon and Creswell,<sup>1</sup> in a thorough clinical study of this problem, showed that in a group of 556 giving a history of previous toxin-antitoxin immunization, the incidence of serum reactions after subsequent therapeutic serum injection was 7±1 per cent, whereas, in a group of 151 with a history of having received therapeutic serum only, the incidence was 43 per cent, while of 1750 in a control group who had never received any form of serum, the incidence was 16 per cent. They also found that reactions from therapeutic serum given after toxin-antitoxin immunization were generally more severe and included more immediate types of reactions than occurred in either the control group or in the group with a history of previous therapeutic serum administration. These observations fully corroborate my own experience as summarized in a clinical and laboratory study of serum sensitiveness<sup>2</sup> after toxin-antitoxin published in the March issue of the *Journal of Allergy* in which in summary it was found that horse serum containing toxin antitoxin mixtures definitely sensitized 27.9 per cent of children to horse serum, that this sensitiveness is not limited to the skin alone but involves other tissues as well, that it is influenced by allergic predisposition and finally, that reinjection of immune sera containing larger amounts of horse serum into such individuals may be productive of quite severe serum reactions.

Because of these facts, it seems quite important and highly advisable to employ a means of preliminary testing which will pick out, if possible, those cases in which serum reactions are more likely to occur and in which special care should be taken in the administration. Up until recently, in addition to an inquiry into the history for evidence of allergy either in the patient or his family, the test most frequently used consisted in the intradermal injection of a small amount of the immune serum or of normal horse serum either concentrated or diluted. A positive reaction indicated by an increase in the size of a wheal together with a surrounding area of redness or erythema, was considered evidence of serum sensitiveness and the serum either withheld or given with great care. It has been thought by some workers, particularly by Paik<sup>3</sup> and his co-

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workers, that positive skin tests did not necessarily indicate tissue sensitiveness, that the skin was too sensitive and therefore not a good index of a patient's possible reaction to serum injections. In 1928, Spicer<sup>1</sup> as a result of a comparative study of the intradermal and ophthalmic tests to horse serum, concluded that from the standpoint of serum reactions, the ophthalmic test, although less sensitive, was a much better criterion and should be used in preference to the skin test.

Critical analysis of this study reveals the fact that although in the cases in which the preliminary ophthalmic test was positive, serum reactions were more severe and more frequent, nevertheless, in those cases in which the ophthalmic test was negative and the skin test positive, in spite of spaced administration of the serum, serum reactions of a less serious nature occurred, proving apparently that the ophthalmic test did not select all of the possible reactors. Nevertheless, upon the basis of these recommendations, the test has been adopted by many physicians and is being advised by commercial houses as the sole test for sensitivity necessary prior to serum administration.\*

During the past two years in the course of other investigations in connection with serum sickness, I have had an opportunity of comparing the ophthalmic and skin tests in both adults and children and in relation to serum reactions. A portion of these results has been included in the previously mentioned article. This portion, however, refers rather briefly to studies obtained in children only and does not include similar studies done in adults. Because of the importance of the subject in relation to serum administration, it seemed worth while therefore to report these results separately. For purposes of convenience, the studies will be considered in two groups: (1) among children and (2) among adults.

*Material*—The first group consisted of 248 children obtained mostly from the wards and Out-Patient Department of the Babies' and Graduate Hospitals with a small group obtained from a near-by orphanage. These children varied in ages from six months to thirteen years. The second or adult group consisted of 107 young normal adults (females) varying in ages from eighteen to twenty-five. Except for control groups of 100 children and 26 adults who had never received serum in any form, the remaining individuals gave a history of some form of administration of horse serum either as diphtheria toxin-antitoxin mixtures or antitoxin or in small doses of scarlet fever antitoxin given intradermally for testing purposes. In all instances the serum administration had occurred more than six months prior to the testing.

*Methods*—As recommended by Spicer the ophthalmic test was carried out by the instillation into the conjunctival sac of one eye of one drop of concentrated horse serum or horse serum diluted 1:1 or 1:2 with normal saline solution, concentrated horse serum being preferred. A positive reaction was

\* After the completion of this article my attention was called to a report published by Claiborn in a recent (May 14, 1932) issue of the J. A. M. A. in which the author concluded from a comparative study of the ophthalmic and skin tests for horse serum sensitivity in relation to serum reaction that the ophthalmic test was of greater clinical value and simpler of performance. In Claiborn's series 44 patients gave a history of previous serum injection. In this group the incidence of serum reactions was considerably higher than in patients with negative history and slightly more in patients with positive skin and negative ophthalmic tests than in patients with both tests positive. As previously mentioned it is in this group of cases that care is necessary in the administration or reinjection of serum, particularly if the serum is of the antistreptococcal or pneumococcal types or if it is to be given in children, since reactions in both these instances are apt to be more severe or even fatal if proper precautions are not taken.

then indicated by the occurrence within twenty minutes subjectively of itching and lachrimation and objectively of injection of the vessels of both the corneal and palpebral conjunctiva, and of edema of the eyelids. Depending upon the degree of vascular injection, the reaction was read as slight, moderate, or marked. At the outset of the studies in children, it was soon found that the instillation of concentrated horse serum was productive in certain instances of rather severe local reactions, so that it became necessary in order to avoid these reactions to resort to a 1:10 dilution of normal horse serum whereas in adults concentrated horse serum was used.

The skin test was carried out by the intradermal injection of an extremely small amount (not more than 0.02 cc) of horse serum diluted 1:10 for adults and 1:100 in children. These dilutions have been found from previous experience in control cases to be entirely satisfactory and not productive of nonspecific positive reactions which occur either after administration of the more concentrated form or of amounts as large as 0.1 cc. Reactions were graded in accordance with the size of the wheal and the area of erythema and listed in accordance with Cooke's classification as slight, moderate, and marked reactions.

### RESULTS

The results are shown in Table I.

*a Children's Group*—The control group of 100 patients showed an extremely small incidence of positive skin reactions, 5 per cent. In only one patient was this reaction at all marked, both in the skin and in the eye tests. This patient, a little colored girl of six, was an allergic patient with early hay

TABLE I  
SUMMARY OF RESULTS OBTAINED

GROUP		TOTAL NO		REACTIONS IN PERCENTAGE			
				NEGATIVE	SLIGHT	MOD- ERATE	MARKED
Control Group	Children	100	Skin test	95.0	4.0	0	1.0
			Eye test	99.0	0	0	1.0
	Adult	26	Skin test	96.0	0	0	4.0
			Eye test	100.0	0	0	0
Test Group	Children	148	Skin test	58.9	14.1	10.8	16.2
			Eye test	85.0	7.0	4.0	4.0
	Adult	81	Skin test	52.5	14.8	23.4	9.3
			Eye test	90.1	6.1	1.2	2.6
Combined Group		229	Skin test	55.3	14.4	15.4	14.9
			Eye test	85.1	7.8	3.0	4.1

fever, but gave no history of previous serum injection, so that the sensitivity was probably of a natural type. The other four positive skin reactions were slight in character. The small percentage of positive reactors in this group in contrast with the somewhat higher percentage of naturally sensitive individuals reported by both Park and Hooker, may be accounted for, perhaps, by the fact that in the present studies a smaller amount of a more dilute solution of horse serum was used for testing instead of the more concentrated forms of either horse serum or serum globulins.



Except for the one marked reaction mentioned above, the remaining 99 per cent of eye reactions in the control group were completely negative.

As a contrast to the control group, of the 148 patients in the test group upon whom both tests were performed, 87 or 58.1 per cent had completely negative skin reactions, 122 or 85 per cent completely negative ophthalmic reactions. Of the 61 with positive reactions, 21 or 34.1 per cent were slight, 16 or 26.1 per cent moderate and 24 or 39.1 per cent marked reactions. Of the 26 with positive ophthalmic reactions, 13 or 50 per cent were slight, 6 or 23 per cent moderate and 7 or 26.1 per cent marked reactions. Complete agreement in both character and degree occurred in only 18 per cent of the positive cases and in only 15 per cent of the entire group. In no instance was a positive ophthalmic reaction obtained in a patient showing a negative skin test.

*Adult Group*—Of the 26 members of the control group, only one showed a positive skin reaction of marked degree but no history of previous serum injection could here be obtained. In none of these was a positive ophthalmic reaction obtained. Of the 81 members of the test group, 43 or 52.5 per cent showed completely negative skin reactions and 38 or 47.5 per cent positive reactions, 12 or 14.8 per cent being slight, 19 or 23.4 per cent moderate and 7 or 9.3 per cent marked in character. As a contrast, the ophthalmic reaction was negative in 73 or 90.1 per cent, slightly positive in 5 or 6.1 per cent, moderate in 1 or 1.2 per cent and marked in 2 or 2.6 per cent. Agreement occurred therefore in 10 per cent and disagreement in 90 per cent. In three instances in adults in which the immediate eye reaction was negative, delayed reactions occurred, one being of rather marked character with considerable conjunctivitis lasting two days. Delayed skin reactions occurred in only two instances but were not attended by any particular discomfort.

*Comment*—If the figures obtained for both groups be combined, it will thus be seen that in the control group of 126, the skin reaction was positive in 4 per cent and the ophthalmic reaction in 0.8 per cent. In the test groups, the combined total positive skin reactions was 44.7 per cent, whereas, that for the ophthalmic reaction was 15 per cent. If, therefore, one is to be guided by the ophthalmic reaction alone, sensitiveness to horse serum should be detectable by means of this reaction, in only 15 per cent of cases, whereas with the skin test the chance of detection would be three times as great. Admittedly, therefore, the skin test is much the more sensitive of the two reactions and since, as stated at the outset, the increased frequency of serum reactions makes it advisable to employ as sensitive a test as possible, from the standpoint of sensitiveness, the skin test would seem the more preferable of the two. While in patients with a negative serum history, the ophthalmic test might be relied upon to detect the possible reactors, it is not the test of choice in patients with a history of previous serum administration no matter how small an amount this may be.

Besides the fact of its diminished sensitiveness, the eye test in my experience was found to possess other disadvantages rendering it unfit for general clinical use. It is practically valueless in crying children, first, because one could not be sure that the test solution was not washed out by the tears, and second, because the congestion of the conjunctiva caused by the crying made it difficult to read a positive reaction. Furthermore, a markedly positive reaction was followed in

some instances by marked swelling of the lower eyelid and while this was controlled readily by the instillation of a drop of adrenalin 1:1000 into the conjunctival sac, the reaction when present was quite annoying to the patient and alarming to the parents, especially if the swelling persisted several days, as occurred in one patient. On the other hand in the dosage and dilution used, the skin test was never productive of any discomforting reactions. Because of the disadvantages, and since the necessity for serum administration occurs more frequently in children than in adults, I believe it inadvisable to rely upon the ophthalmic reaction alone as a criterion of serum sensitiveness and certainly not in preference to the skin test. It may, however, prove of value in determining the degree of sensitiveness in patients giving moderate or marked skin reactions, in such instances the presence of a positive eye reaction of similar intensity to the skin reaction indicates a high degree of sensitiveness, and therefore that in such a patient serum administration may be productive of an extremely severe reaction.

#### SUMMARY

From a comparative study of the ophthalmic and skin tests for sensitiveness to horse serum, it would appear that the skin reaction is much more sensitive, less annoying to the patient and not possessed of as many disadvantages as the ophthalmic reaction particularly in children, in whom the necessity for serum administration occurs more frequently than in adults.

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# LABORATORY METHODS

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## PICTORIAL AND PLASTIC MEDICAL ILLUSTRATIONS

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IT OFTEN becomes a problem for the physician to determine the best way to illustrate a case for medical publication or for reporting at a medical meeting. The information that he really wants is: What medium of illustration will show his case to the best advantage so that it will be easily and clearly understood by his colleagues.

For this purpose the physician has at his disposal three mediums of illustration, provided that he knows where and how to obtain them. The first of these is photography, the second is drawing or painting, and the third is modeling or sculpture. For the purposes of medicine, photography is used more than drawing, and drawing is used more than modeling.

There are some cases in which the photograph will serve as a better illustration than the drawing, and other cases in which the drawing is indispensable. *The medium of illustration to be chosen depends entirely on the subject to be illustrated and for what purpose the illustration is to be used.* The photograph is the cheaper method and takes less time to produce. Naturally it is the most popular method of picture making. But there are cases in which it is absolutely impossible for the photograph to show to a good advantage in comparison with the drawing. There are also times in which the making of a drawing would be an unnecessary expense and waste of time when the photograph would be sufficient.

Examples of such cases where the ordinary photograph is suitable are as follows: skin lesions in which color is of little importance, deformities of any part of the body that manifest themselves on the outer surfaces, pathologic specimens void of color as after fixation in formalin, apparatus of various types such as chemical, surgical, and medical. In Fig 1, such a photograph illustrates the type of case that is well adaptable to photography as a medium of illustration.

In many cases pen and ink diagrammatic line drawings of apparatus show to a better advantage how such equipment is operated.

There are cases in which the best photograph is worthless in comparison to the drawing made by the finished medical artist. It is not only hazardous,

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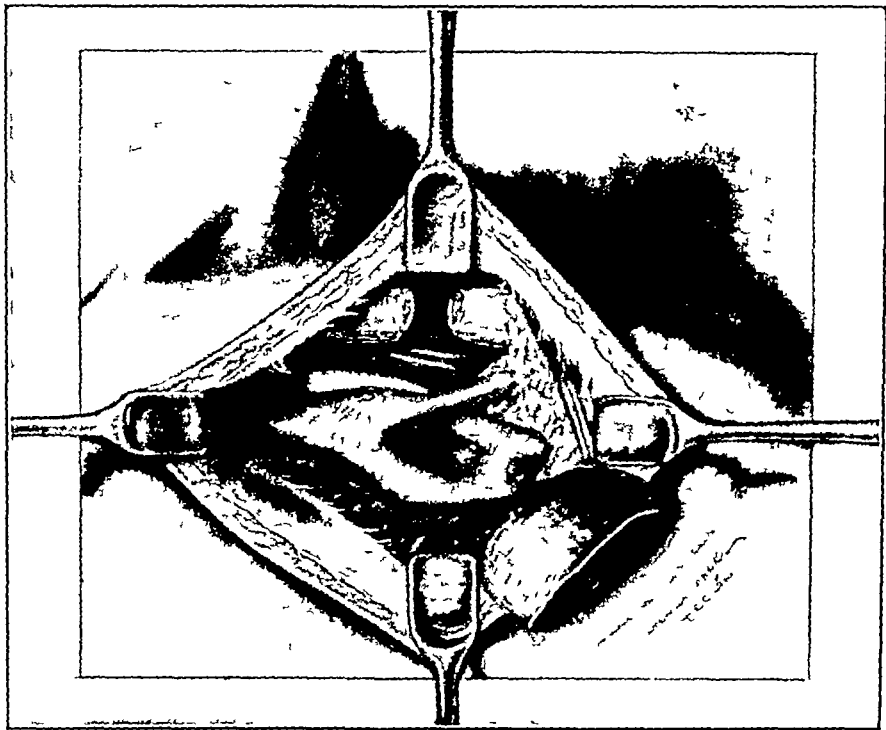


Fig 1—Multiple abscesses of brain A typical photograph of a pathologic specimen

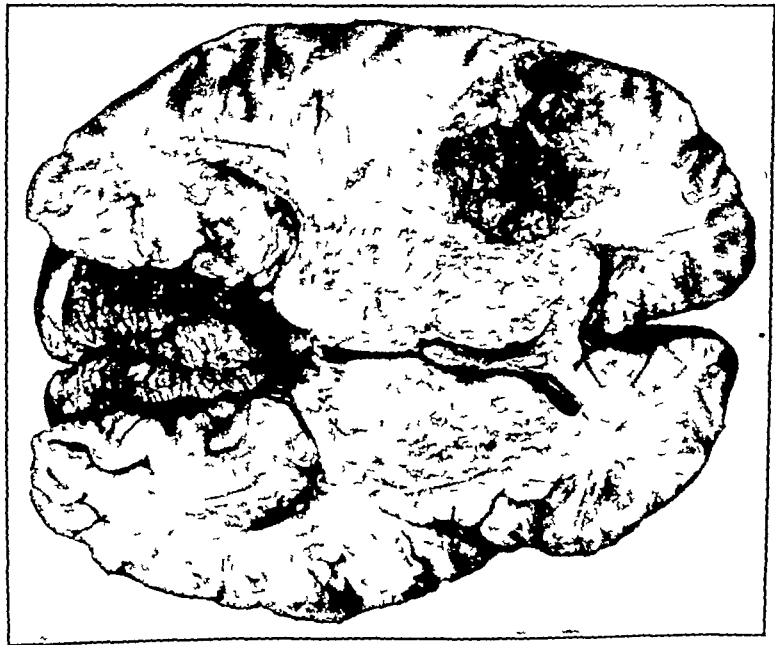


Fig 2—Arterio venous aneurysm A typical surgical drawing

but practically impossible, to obtain a photograph through the small incision that is made to gain access to the abdominal and thoracic viscera. Even the most proficient photographer, using the best equipment, is apt to fail or refuse to try. Without doubt the mechanics of surgery (Fig 2) are best illustrated through the medium of draftsmanship. Even the simple pen and ink diagram is often superior to the most perfected photograph in presenting a surgical procedure for mental absorption.

In these cases drawings are superior not only because of the inaccessibility of the field but also because important structures are often covered by blood, mucus, fascia, fat, and other unimportant parts that prevent a clear view to the lens of the camera. The artist can eliminate these obstructions from his drawing while it is generally impossible for the photographer to do so in his photograph. Of course photographs can be retouched, but when any great amount of retouching is done, the photograph becomes more of an art job than a photograph. In the end it would have been better for the artist to have made the entire illustration.

As photographs are seldom satisfactory for illustrative purposes when made through small incisions, they are also at the present time of little value when made of such subjects as the vaginal and rectal walls and the interior of the ear, nose, and mouth (Fig 3-A). I believe that a technique can be developed for doing this work. It will depend largely on the proper lens system and proper lighting effects. At the present, in such cases as mentioned above, the artist can produce the better illustration.

When numerous blocks have been cut from pathologic specimens for making microscopic sections, it is best to turn the specimen over to the artist to make a reconstructed drawing (Fig 3-B), while on the other hand if the blocks have done little toward mutilating the specimen, it may be photographed.

It is indeed hard for the photographer to make perfect "ghost" pictures (Fig 4) that can be used for medical illustration. A "ghost" picture is produced by superimposing one subject on top of another so that both subjects can be clearly seen. An example is the superimposing of transparent flesh over a bony structure so that the contours and outlines of both flesh and the bones can be seen clearly in a single picture. The artist can draw organs of the abdominal cavity as though they were made of transparent glass so that the parts in a posterior position can be seen clearly. The relation and position of one part to another is thereby observed in a simplified manner.

It is also possible for the photographer to do this by the process of double exposure, but in most cases the result would be faulty in the anatomical relation of parts. A good drawing of such a subject would be far superior. There have been, however, many "ghost" photographs made of cases of ankylosis, showing by double exposure the extent of movement of the extremities.

In making a comparison between the drawing and the photograph made through the microscope it often becomes a matter of personal taste on the part of the physician as to his preference. The author believes that the average physician is more accustomed to poor photomicrographs than he is to



Fig 3-A —Epulis

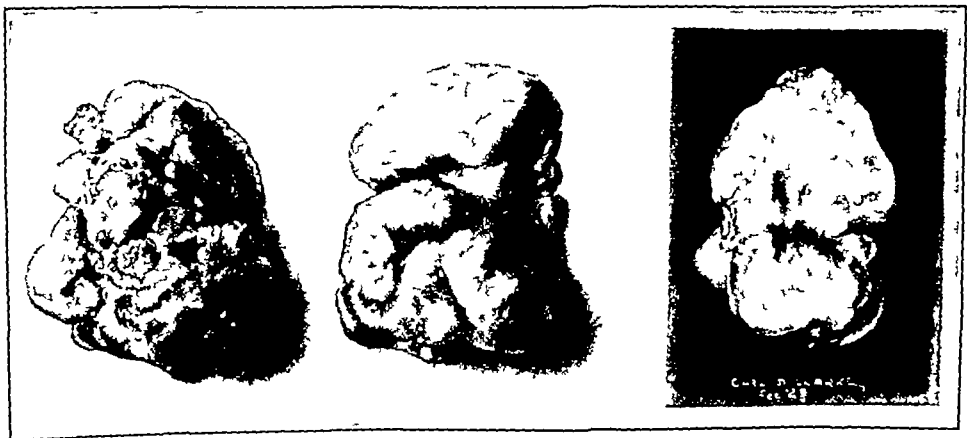


Fig 3-B —The tumor as seen in Fig 3-A after removal showing anterior and posterior views and cross-section. The actual specimen was cut for making microscopic sections. This is a reconstruction of the tumor as seen before sectioning.

good ones. Not all physicians are lucky enough to have at their disposal trained medical artists and photographers. In fact the making of medical pictures is often left to artists and photographers doing the usual run of commercial work. Few if any commercial photographers have the equipment for making photomicrographs. Since this is a highly developed and specialized field of photography it is also assumed that few commercial pho-

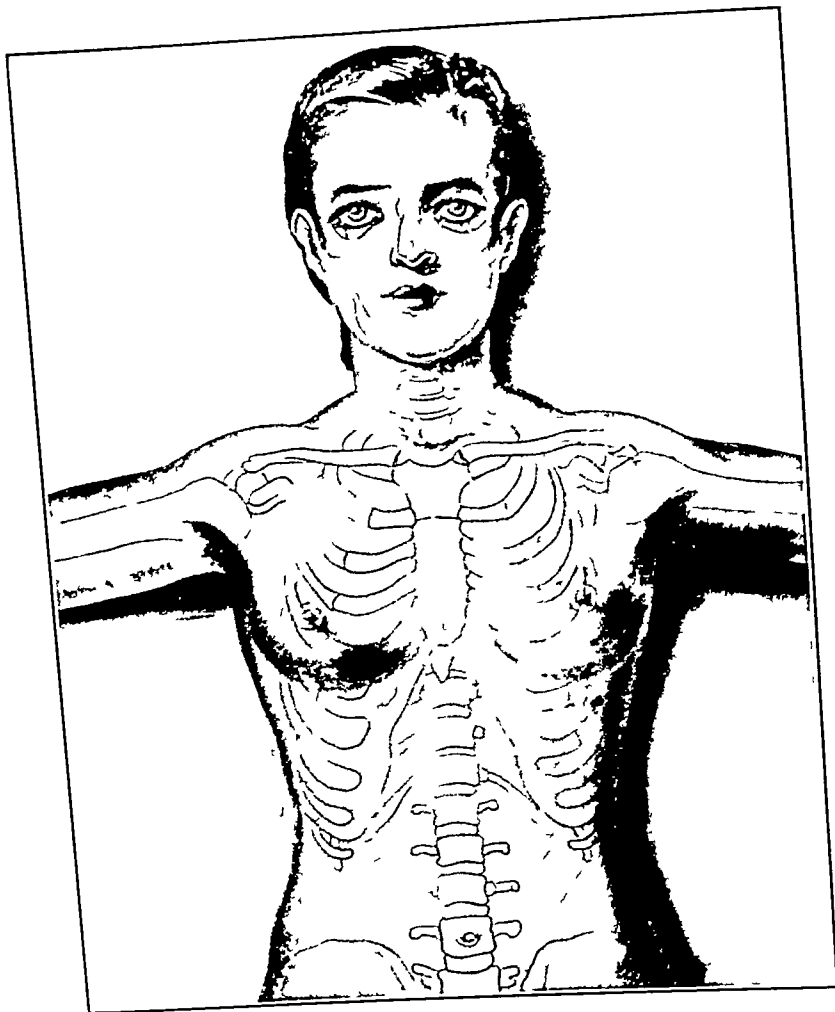


Fig. 4.—A ghost picture. Photographs of this drawing were made to show the various incisions for the removal of the breast, each incision being drawn on a separate photograph.

tographers understand how to obtain the best results. Therefore photography of this type is left to the pathologist or laboratory worker, who may be very proficient in his own field but a poor photographer. He may consider his results good until a comparison with truly excellent photomicrographs is made. Fig. 5-A is an example of an excellent low power photomicrograph while Fig. 5-B is a good example of a photograph taken through the oil immersion lens and compensating eye piece.

The photomicrograph of some years back was a poor excuse for a good drawing done at the same time of the same subject. With the continued development and use of panchromatic photographic plates, color screens, apochromatic lenses, and perfected photomicrographic apparatus, the drawing



Fig 5-A—Microscopic section of bone. A typical low power photomicrograph

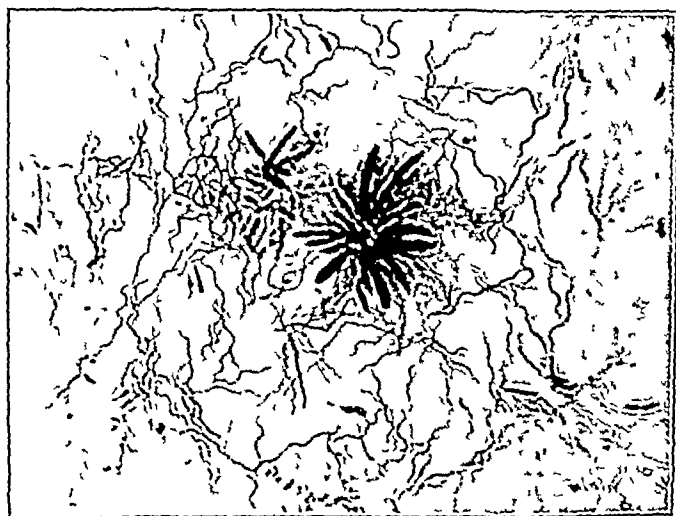


Fig 5-B—*Leptothrix falciiformis* *Spirocheta vincentii*. A typical high power photomicrograph of the microscopic subject is beginning to recede into the background. Photomicrographs of any microscopic section can be made through any known and used power of lenses. In other words, low power pictures covering the entire section can be produced as well as the pictures made through the oil



immersion lens. Any degree of magnification between these two can be successfully obtained and the section photographed at that specified power.

It is possible for the artist to accentuate any part of the cell structure as well as "hold back" the insignificant parts in a microscopic slide. The pho-

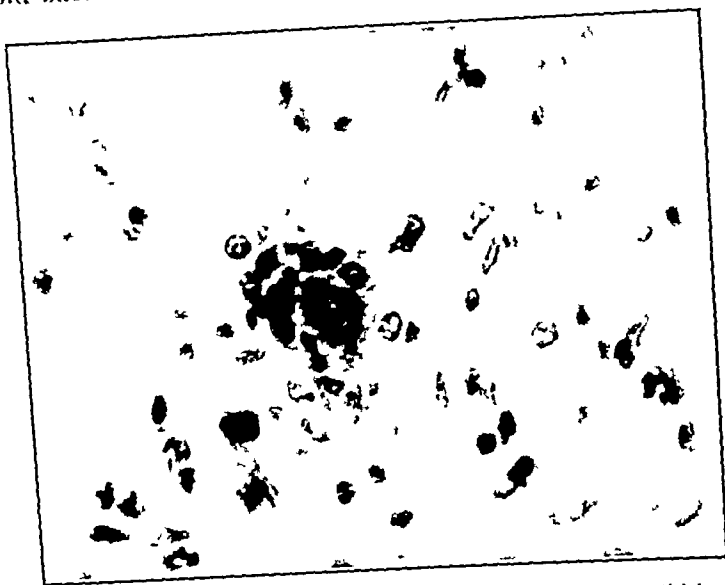


Fig. 6-A.—Pollomycitils (monkey's brain) Nissl preparation 20 mm thick. Compare this photomicrograph with the drawing in Fig. 6-B.

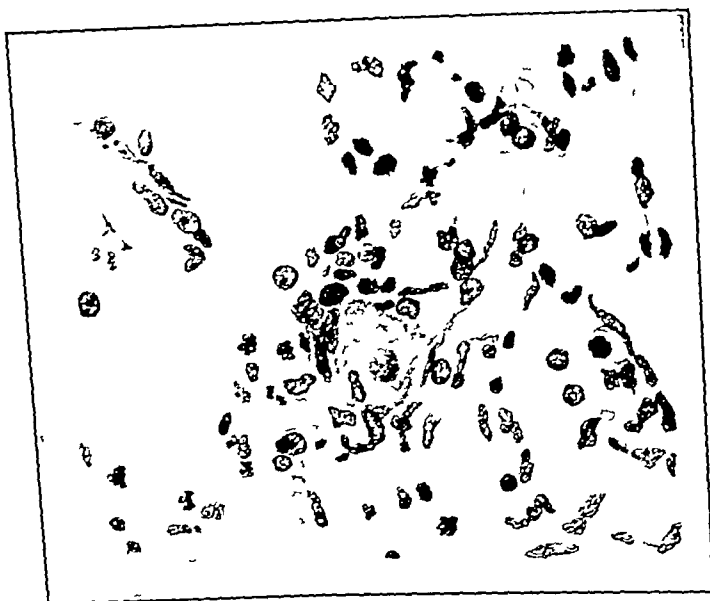


Fig. 6-B.—Drawing made through the microscope of the same section as seen in Fig. 6-A.

tographer can do the same thing by the use of the proper color screen or screens with the proper plate and slight variations in exposure and development of the negative and positive. It stands to reason that the photograph is a truer representation of the original. However, in high power work, detail

and depth in a cell is often hard to obtain by photography. The artist can improve on some photomicrographs because he can make a composite picture of what comes in and out of view as he focuses his microscope up and down. Fig 6-A is a high power photograph that is good as far as the photographic technique is concerned, but the drawing, Fig 6-B, of the same subject is superior, due to this ability of the eye and hand to record on paper in a single picture the various depths of a microscopic section.

It is possible to make natural color photographs that can be used as lantern slides or for publication. These pictures in color are made on glass or celluloid film and are often exceptionally well done. As yet, the average natural color photograph does not compare favorably with the average color drawing used in medical publications.

At present no satisfactory process has been perfected for producing a photographic picture in natural color on photographic paper suitable for medical illustration.

It is possible for the artist to color or tint photographs but the drawing or painting by the artist without photographic aid is generally superior. Photographs to be colored or tinted as medical illustrations should first be made in sepia tone. This is necessary, as the black undertone has a tendency to "kill" or destroy the brilliancy and strength of warm colors such as reds and yellows. These colors are used in painting or glazing over the photograph.

One of the important factors to be considered before having full tone colored pictures made is the expense of reproduction. This expense is augmented by the necessity of making numerous photoengraved cuts and the added processes of painting. Many medical journals require the author or his institution to bear this expense for color reproduction. However, the cost of full tone color pictures is greatly reduced by using flat color only. By the use of flat color over black and white halftones or line drawings, such structures as veins, nerves and arteries, etc., can be accentuated. The color illustrations in the "Hand Atlas of Human Anatomy" by Spalteholz are examples of the use of flat color in photoengraving. The average magazine cover is generally reproduced in full tone.

It is the author's general assumption that better reproductions in black and white are obtained from photographs or drawings that are themselves black and white rather than in color. Therefore, if the picture is not to be reproduced in color, have the artist or photographer make the original in black and white.

Practically all lantern slides are made by the photographic process, no matter what the original may be. One exception to this rule is when the diagram or sketch is drawn directly on a prepared glass of lantern slide size, another is the process of typewriting through carbon tissue placed next to a piece of cellophane or celluloid. This latter method gives a carbon impression on the cellophane which is mounted between two lantern slide cover glasses for projection.

Wax or plaster models can be made of skin lesions, deformities and abnormal growths and depressions of the body. These models or casts are made by first applying liquid plaster of Paris to the greased surface. When this

plaster has set and removed it constitutes the *mould* which in turn is filled with plaster or wax. The mould is removed after the hardening of the plaster or wax, thereby giving a cast in detail, even showing the pores of the skin, provided that the work is properly done.

There has been a new process of casting devised called the "Poller Method" which is far superior to the old plaster procedure. This new method employs the use of a hydrocolloidal wax-like substance that is pliable enough to permit free removal of the mould from the anatomical part in cases where there is a degree of under cutting. The mould may be filled with wax or plaster to obtain the positive.

In this way, life-like reproductions can be made of the extremities, skin conditions, or any part of the abdominal or thoracic viscera after removal from the body. These casts can be colored to represent the originals and have often been mistaken for the actual organ or part.

Cut or destroyed parts of specimens can be reconstructed before casting. If the worker is clever, he can model or sculpt the entire subject in either clay, wax, or plasteline. From his original work a *piece mould* can be made to reproduce a number of casts in colored wax or plaster. A limited number of casts can also be made from a Poller mould.

These casts are considered to be the best representation of the original but are cumbersome to handle in exhibiting as compared to the photograph or drawing. Photographs and lantern slides can be made from them for reproduction or projection. Wax or plaster casts serve best as museum pieces. They can also be used for student instruction in small groups.

In conclusion, it may be repeated that the subject to be depicted and the purpose for which the illustration or reproduction is to be used determine the medium through which it should be produced. It will be well worth the physician's time to discuss this matter fully with the artist and photographer before he blindly orders a paper to be illustrated. It is hoped that such a discussion will improve medical illustrations and then worth in conveying ideas from one physician to another.

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In an effort to prepare a bibliography on this subject the author had the names and data on about one thousand articles typed in file card form from the *Index Medicus*. While many of the articles described the use of photography in relation to medicine and a lesser number described the use of medical drawings and casts none made the comparisons of one with the other, describing just when a drawing should be made and when the photograph would be satisfactory. For this reason the author believes that such a comparison as has been described will be of definite benefit to physicians who write papers and report cases at medical gatherings.

Those who care to review the list of articles that were compiled from the *Index Medicus* may do so in the March, 1933, issue of the *Journal of the Biological Photographic Association*, a copy of which may be obtained from the editorial office at 519 West Lombard Street, Baltimore, Maryland, or from the more up to date medical libraries.

## SPOT METHODS IN THE DETERMINATION OF $P_H$ \*

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ALTHOUGH spot methods for determining  $P_H$  have been in use for a number of years in many laboratories, factories, etc., such procedures have probably been employed more extensively in clinical and bacteriologic work than elsewhere. This is no doubt due to the fact that the methods require so little material. In view of this it seems advisable to examine the various procedures critically, pointing out their advantages, disadvantages, and possible sources of error.

The general procedure is to place a small amount of the fluid to be tested, usually measured by drops, on an ordinary laboratory spot plate, a nonsoluble plate of opal glass, or in a movable glass cell on a white background. A small amount of indicator solution is then mixed with the sample and the resulting color compared with color standards. Color standards may be ordinary printed color charts such as are found in "The Determination of Hydrogen Ions," by W. Mansfield Clark, tinted celluloid disks, vitrified colored porcelain or the standards may be prepared as required from buffer solutions which have previously been standardized by a potentiometric method.

Of the procedures employing buffer solutions as standards those of Felton<sup>5</sup> and Brown<sup>3</sup> have been used extensively. In Felton's method a drop of the solution to be examined is mixed with a drop of the proper indicator solution on a piece of alkali-free opal glass. Around this are placed drops of standard buffer solutions and with each drop of buffer solution is mixed a drop of the same indicator solution used with the unknown. The spots thus produced will exhibit colors varying in shade with their  $P_H$  and by carefully matching the color of the unknown solution with the known buffer spots, the  $P_H$  value may be ascertained.

The method of Brown employs small glass cells, approximately 10 mm inside diameter and 3 mm deep, holding about 0.25 cc or 5 to 7 drops of water from an ordinary dropping pipette. When buffer solutions in intervals of 0.2  $P_H$  are employed, the color standards are prepared by filling each of nine cells with the proper buffer solutions and a drop of the proper indicator solution. A  $P_H$  determination is made by filling another cell with the sample and a drop of the same indicator solution, and comparing the color with that of the standards. Comparison is made on a milk-white glass plate. The use of cells provides uniform depths and consequently uniform color fields. Alcoholic solutions of the indicators are employed so that the diffusion currents will cause mixing.

The advantages of spot methods in general are simplicity (omitting the standardization), rapidity, and economy of materials. They lend themselves

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readily to situations where high accuracy is not required and can frequently be used when only small amounts of material are available. However spot methods have many disadvantages some of which are easily discernible, while others are not so apparent. The relative accuracy of such methods may vary enormously with the nature of the material being tested and they should never be relied upon unless all the possible sources of error have been investigated.

Irrespective of the nature of the material under examination, the standardization must be correct. The color standards must represent the proper values and they must give colors that are reasonably true. If the standards, whether printed charts or buffer mixtures, are incorrect or become incorrect in time, the procedure then becomes a mere approximation.

Probably the fairest evaluation of printed color charts is that given by Clark in discussing the value of the chart found in his book. He states, 'Aside from the inherent difficulty in fixing a printed color from the effect of the vehicle, there remains the utter impossibility of reproducing with paper and ink the effect observed in a liquid solution. The fundamental phenomena are quantitatively very different in the two cases. Therefore, the user of the chart of colors will have to use discretion and some imagination. If he does not attempt to make the reproduction take the place of the standards, he should find them useful for classroom demonstrations, for refreshing the memory and for rough standards.' In addition all printed or tinted color charts may fade in time.

The reliability of buffer solutions as standards naturally depends on their stability. It is a mistake to assume that any buffer solution will retain its original  $P_H$  value for any appreciable length of time when it is intermittently exposed to the atmosphere as it must be when in use. The action of organisms, acid or alkaline fumes and even the normal carbon dioxide content of the air may appreciably modify the  $P_H$  of such a mixture. The action of the carbon dioxide in the air is of course most noticeable on alkaline buffers.

In Table I are given some data showing the changes that took place in a series of buffer solutions which were being used in the method of Brown. These solutions were originally carefully standardized by a potentiometric method, placed in Pyrex glass vials closed with pipettes and rubber nipples. The solu-

TABLE I  
CHANGES IN  $P_H$  OF BUFFER SOLUTIONS USED IN BROWN'S METHOD

INITIAL $P_H$	$P_H$ AFTER THREE MONTHS	INITIAL $P_H$	$P_H$ AFTER THREE MONTHS
10.0	9.8	7.8	7.4
9.8	9.6	7.6	7.3
9.6	9.5	7.4	7.3
9.4	9.3	7.2	7.1
9.2	9.1	7.0	6.9
9.0	9.0	6.8	6.7
8.8	8.7	6.6	6.5
8.6	8.4	6.4	6.3
8.4	8.2	6.2	6.1
8.2	7.8	6.0	5.9
8.0	7.7	5.8	5.7

tions were used about once each week and care was taken not to contaminate them in any way. After three months they were again checked potentiometrically. All measurements were made at 25° C.

Although most of the solutions changed in  $P_H$  value, there was no evidence of molds, etc., in any case. These changes are about what can be expected under normal conditions and are what the author has found to be general during some seven years' experience with the method. In cases where molds develop the changes may of course be much greater.

The buffer solutions reported in the table were made in accordance with the data given by Clark except that in the boric acid-potassium chloride-sodium hydroxide series,  $P_H$  7.8-10.0, it was found necessary to add more M/5 sodium hydroxide solution in each case than called for in Clark's table. Others have also found it necessary to increase the added alkali in order to secure solutions of the desired  $P_H$  values.<sup>2</sup> This may be due in part to the fact that Clark standardized at 20° C.

Alkaline buffer solutions which are stable to an exposure have been prepared by Acree and Fawcett.<sup>3</sup> While such mixtures are not influenced by the carbon dioxide of the air, they are not stable against laboratory fumes, etc. Although mixtures can be made sterile in sealed containers, they are subject to contamination as soon as they are opened for use.

Mold growth in buffer solutions can be controlled to a considerable extent by the addition of a preservative. The preservative ordinarily added to commercial buffer solutions is thymol and if a crystal of this substance be kept in the solution mold growth will be inhibited. Solid thymol must always be present to maintain saturation. The addition of thymol has some disadvantages. Its presence may cause difficulty if one wishes to check the  $P_H$  by means of a hydrogen electrode. It does not appear to affect the quinhydrone electrode. Acid buffer solutions ( $P_H$  1.0-2.2), which have been saturated with thymol, will usually show off colors (bluish) on the addition of the indicator, thymol blue. This is apparently a colloidal effect.

Another possible source of change in the buffer solutions when pipettes and nipples are kept in each vial or bottle is in returning a pipette to the wrong container. This marks a careless technician but it sometimes occurs. To avoid such mistakes only one pipette should be removed from its container at a time.

The safest way to avoid errors due to deterioration or contamination of buffer solutions is to check the solutions often by some accurate method. A hydrogen electrode or colorimetric outfit employing permanent standards can be used. Monthly checking or renewal is advisable.

One of the outstanding defects in any spot method is that there is no practical way to compensate for color and turbidity in the sample except by dilution. This limits the application to colored or turbid materials which show no appreciable change in  $P_H$  on moderate dilution, i.e. to highly buffered substances. It must be admitted, however, that experienced workers can often estimate the  $P_H$  closely even when color and turbidity prevent exact color matches. In spite of this off colors in the sample due to even small amounts of color and turbidity are

always potential sources of error. Errors are more liable to be made when the colors encountered are similar in quality to those imparted by the indicator. Fair color matches can then be secured even when the readings are in error, whereas if the colors are pronouncedly different, no satisfactory match can be secured and the method is abandoned as inapplicable.

Another defect in the spot method lies in the fact that the sample is unduly exposed to the atmosphere during the test. Consequently the chance of absorbing carbon dioxide or in some cases of evolving it are very high. In materials that are only slightly buffered this may result in rapid changes in the  $P_H$  of the sample before a reading can be made. Distilled water in equilibrium with the normal carbon dioxide content of the air will have a  $P_H$  of 5.7 to 5.8. If the water is passed through a special still or boiled in a Pyrex glass vessel, most of the carbon dioxide can be removed to yield water with  $P_H$  6.6 to 6.8. When a sample of distilled water showing  $P_H$  6.8 by the isohydric indicator method of Acec and Fawcett<sup>1</sup> is tested by the method of Brown, the  $P_H$  of the test portion drops below 6.0 in a very few seconds. Obviously no satisfactory results can be secured under such circumstances. The case of distilled water is of course an extreme one since the water is practically devoid of buffer action. However, in all materials of low buffer content there is a tendency for the  $P_H$  to change during the test and this renders any spot method of dubious value in such cases.

Loss of carbon dioxide during testing is another possible source of error when using spot methods. In shallow layers the tendency for carbon dioxide to escape is more pronounced than in deep tubes such as are used in comparator methods. For example a sample of saliva will show an increase in  $P_H$  on standing due to this tendency.

Although it has been demonstrated repeatedly (see references by Acec and Fawcett<sup>1</sup>) that in determining the  $P_H$  of slightly buffered materials, the  $P_H$  of the indicator solution must be fairly close to that of the sample, little attention has been paid to this factor in most spot methods. When the proportion of indicator solution used is large as in the method of Felton, the  $P_H$  of the indicator may have enormous influence on the results even when the sample has some buffer action. In addition the alcoholic solutions used in the method of Brown, are invariably more difficult to keep adjusted than are aqueous solutions.

In spite of the fact that spot methods have been devised primarily for determining the  $P_H$  of materials that are fairly well buffered and moderately clear and colorless, there has been a tendency to apply them to numerous situations in which they are entirely inapplicable. This is probably due to their convenience and also often to the commercial availability of equipment using such methods. In fairness to those who have introduced some of these procedures it should be said that they can hardly be held responsible for the indiscriminate use of such methods. They have invariably stated the limitations of the methods and described the nature of the materials on which they were able to secure satisfactory results.

## CONCLUSIONS

Although spot methods for determining  $P_H$  are convenient, rapid, and economical in material, such methods should never be used indiscriminately.

Careful check should always be maintained on all standards, whether color charts or buffer solutions are used.

Spot methods should be used with extreme caution on all colored and turbid materials and on all samples not known to be fairly well buffered.

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## A SEMIMICROMETHOD FOR THE ANALYSIS OF SULPHUR IN FINGER NAILS\*

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SULLIVAN and Hess<sup>1</sup> recently reported on the lowered cystine content of finger nails in certain cases of arthritis. An investigation by Thomas F. Wheeldon, Roland J. Mann and William A. Peabody (in progress) aims to determine (1) the value and limitations of colloidal sulphur therapy in arthritis, and (2) the extent to which nail analyses may contribute to diagnosis, prognosis, and the study of the effects of sulphur treatment. In the course of this work, we felt the need of a rapid and consistent method of analysis. Inasmuch as the total sulphur of nails is practically all in the form of cystine, the latter may be determined indirectly by any method suitable for total sulphur.

The Benedict-This-benzidine procedure seemed unsuitable because of the time necessary for oxidation and the small amount of sample usually available. Attempts to use aqueous alkali decomposition followed by recovery as  $CuS$  and microtitration of the  $Cu$  by thiosulphate gave encouraging results with cow horn when a little glucose was used to minimize oxidation during decomposition. But with human nails the procedure proved too inconsistent, sometimes accurate, occasionally high (due to nonsulphide  $Cu$ ?), more often low, probably due to incomplete decomposition or precipitation, to partial conversion of sulphide to other forms, or to a combination of these factors. With

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reference to alkali decomposition, Thor and Gortner<sup>3</sup> recently have shown that cystine is more stable than had been supposed. While it is likely that the recently described method of Morgulis and Humphill<sup>1</sup> for S in urine could have been adapted to our purpose, the present procedure was designed specifically for nails when the details of the former were unavailable.

The method finally adopted, based upon the barium titration of Balachowski,<sup>4</sup> consists essentially of an open dry peroxide fusion, precipitation as  $\text{BaSO}_4$ , under specified conditions, and macrotitration of the residual  $\text{BaCl}_2$  with potassium dichromate, with phenol red as indicator. The slight alkalinity of excess dichromate effects the endpoint. Though the detailed procedure appears long, a series of six or more samples can be run in an average time per sample of fifteen to twenty minutes from beginning of fusions to end of titrations. Because of the difficulty of accurately describing the  $\text{PH}$  adjustments and the final endpoint, the analyst is advised to practice with standard sulphate solutions before attempting the complete analysis. Table I indicates the degree

TABLE I

SAMPLE NO	NATURE	CYSTINE PER CENT	
		PRESENT METHOD*	OTHER METHODS
1	Cow horn	9.91, 9.78	{ 9.69, 9.64 <sup>a</sup> 9.97, 9.70, 10.1 <sup>b</sup>
2	Cystine, pure	99.7	
3	† Normal finger nail, subject P	11.4, 11.4	
4	Normal toe nail, subject P	9.81, 9.85	
5	Normal finger nail, subject F	11.7	
6	Normal toe nail, subject F	11.0, 10.7	
7	Arthritis toe nail, subject D	8.83, 8.93	
8	Arthritis toe nail, subject B	8.69, 8.83	
9	Arthritis finger nail, subject N	12.1, 11.6	
10	Arthritis toe nail, subject N	9.68, 9.66	
11	Normal finger nail, subject RF	11.7, 11.6	
12	Normal finger nail, subject KF	12.0, 12.2	
13	Normal toe nail, subject KF	8.90, 8.90	

\* (Benedict-Thies benzidine titration)

<sup>1</sup> (Alkali Cu thiosulphate titration)

\* All samples by this method 25 mg. or less

† Normal subjects not examined clinically

of accuracy of the method and the average run of variation between duplicates. For sample No. 2, the amount of cystine weighed out was unknown to the analyst, within 10 mg. A more extensive series of values for normal nails will be reported elsewhere later (by Wheeldon et al.) with the clinical data. To date the method has given values for normal finger nails which fall within the normal range of 11 to 13 per cent, established by Sullivan and Hess<sup>1</sup> by means of the Sullivan colorimetric cystine method.

#### SOLUTIONS REQUIRED

**Standard Barium Chloride**—1 *Stock solution*. Weigh out about 12 gm. of the C. P. crystalline salt for each liter of aqueous solution (approximately 0.05 M). Standardize an aliquot gravimetrically by means of the sulphate, then dilute a suitable quantity to exactly 0.0416 M, to make 1 cc. equivalent to 5 mg. of cystine.

2 *Precipitating solution*. Dilute 1 volume of stock to 5 volumes (5 cc. then equivalent to 5 mg. of cystine).

**Standard Dichromate**—Approximately 0.010-0.011 M solution of C. P. potassium

dichromate Standardize against the dilute  $\text{BaCl}_2$  solution to 5 cc of the latter add 5 drops of the indicator and follow paragraphs 5 and 6 of the procedure

*Special Alkali Solution*—Saturate approximately 0.4 N NaOH solution with  $\text{Ca}(\text{OH})_2$ , store with excess  $\text{Ca}(\text{OH})_2$  in a bottle fitted with a siphon so as to deliver an almost clear liquid

*Phenol Red Indicator*—Phenolsulphonephthalein, 0.05 per cent aqueous

Ethanol, 95 per cent, hydrochloric acids 1.1 and 0.01 N approximately

#### DIRECTIONS

1 Cut the nails with scissors to a size preferably not over 1 mm Weigh about 25 mg accurately on a sensitive analytical balance or a micro balance Collect the sample compactly in the bottom of a 20 cc pure nickel crucible, cover with 300 to 500 mg of sulphur free  $\text{Na}_2\text{O}_2$ , and heat cautiously over a small flame at a rate rapid enough to prevent "balling" of the peroxide but not so rapid as to cause flaming of the sample Fuse completely (about thirty seconds heating after a melt is obtained) Cool partially, add 8 to 10 cc of distilled water, and heat to dissolve more rapidly

Certain lots of  $\text{Na}_2\text{O}_2$  may spit and disintegrate badly in spite of all precautions during heating This behavior can be overcome by first fusing the nails well with 2 pellets (about 350 mg) of NaOH, then oxidizing with not more than 250 mg of sodium peroxide, which thus produces a quiet melt

2 Filter through a 7 cm Whatman No. 40 paper into a 50 cc beaker, wash paper with 12 to 15 cc of water in 6 portions, the first 3 of which have been used to rinse the crucible Evaporate filtrates to about 10 cc

3 Add 5 drops of the phenol red indicator and just enough 1.1 HCl to change the color from pink through yellow to the first appearance of an orange (or yellow with a pink fluorescence)

4 Evaporate carefully to about 7 cc volume, almost neutralize (light yellow) with the special alkali solution, add exactly 5 cc of the  $\text{BaCl}_2$  precipitating solution, and again evaporate to 6 to 7 cc

5 Make slightly alkaline (pink) with the special alkali (1 to 2 drops), add 10 to 15 cc of recently boiled 95 per cent ethanol, bring back to a yellow with approximately 0.01 N HCl Alternately boil and add the dilute HCl until the yellow becomes permanent (one additional drop, or even less, usually suffices)

6 Titrate hot with the standard dichromate from a microburet (the Koch calcium "pipette," 2 cc graduated in 0.01 cc has been found very suitable) The light source should be a frosted electric lamp, shielded, and placed to direct the rays through the beaker horizontally from one side The endpoint is the first definite permanent pink (not recognizable in ordinary daylight)

#### CALCULATIONS

C = mg of cystine (or S) equivalent to  $\text{BaCl}_2$  added

D = dichromate equivalent (in cc) of  $\text{BaCl}_2$  added

$$\frac{(D \text{ Titration}) \times C \times 100}{D \times \text{mg sample}} = \text{per cent cystine (or S)}$$

Example 3.90 cc dichromate = 5 cc  $\text{BaCl}_2$  = 5 mg cystine Wt sample, 25.0 mg ,  
titration, 1.85 cc Calculation  $3.90 - 1.85 = 2.05$   $\frac{2.05 \times 5 \times 100}{3.90 \times 25.0} = 10.5$  per cent cystine

#### PRECAUTIONS, ETC

(1) Smaller samples may be used if necessary, in which case proportionately less  $\text{BaCl}_2$  should be added in Step 4 (3) The first acid evaporation removes  $\text{CO}_2$ , hence the specified acidity should be attained before boiling (4) The  $\text{Ca}(\text{OH})_2$  in the NaOH is used to remove interfering carbonate, silicate, and phosphate from reagent and sample (5) Sufficient addition of 0.01 N HCl is judged by absence of any pink tint when the beaker is viewed against a solid background On the other hand, greater excess of acid than 0.5 drop proportionally

decreases the accuracy of the determination. Some samples of alcohol require prolonged boiling to make them suitable for use in the procedure. (6) In lamplight is described, the final endpoint appears about 0.03 cc sooner and with better definition than in daylight. The standard dichromate solution loses strength very slowly. Weekly restandardization should suffice.

The method has obvious application to the analysis of any sulphur bearing material which is relatively low in phosphite or other interfering union, but we can offer no experience as yet with such application.

#### SUMMARY

A semimicromethod for the analysis of sulphur in human nails is described. The procedure is rapid and accurate for clinical purposes, requires but 25 mg samples, and uses a limited number of pieces of more or less common apparatus.

Acknowledgment is due the Chemical Foundation, Inc., for a grant in aid of this laboratory.

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## AN IMPROVED MULTIPIPETTE FOR ROUTINE QUANTITATIVE DETERMINATION OF ALBUMIN AND SUGAR IN URINE\*

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IN THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE for September, 1927, I described an apparatus which was being used successfully for pipetting urine and reagents in the determination of albumin and sugar quantitatively.<sup>1</sup> The method employed for albumin was a modification of the sulphosaheyleic acid test first described by Folin and Denis<sup>2</sup> and later applied by Kingsbury, Clark, Williams and Post<sup>3</sup> to routine albumin determinations. The test is made by mixing 25 cc of urine with 75 cc of 3 per cent sulphosaheyleic acid and examining for turbidity caused by the presence of albumin at the end of ten minutes. The degree of turbidity is compared with standard tubes of uniform diameter in an illuminated rack with a black background. In this way the amount of albumin can be estimated quantitatively. The Kingsbury-Clark standard tubes employed consist of graded turbidities suspended in gelatin. These are semipermanent and are renewed at the end of about six months. They range in value from 0 to 100 mg per 100 cc and the estimation is reported in mg per cent.

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The sugar method used in this laboratory is that devised by Sumner<sup>4</sup> for normal amounts of sugar in urine. Three cubic centimeters of the reagent which contains dinitrosalicylic acid are mixed with 1 cc of urine and the mixture is placed in a boiling water bath for five minutes, at the end of that time it is cooled, diluted with water to 25 cc, mixed and compared with standards in tubes of equal diameter in a comparator. The presence of sugar produces a reddish brown color, the intensity of which is directly proportional

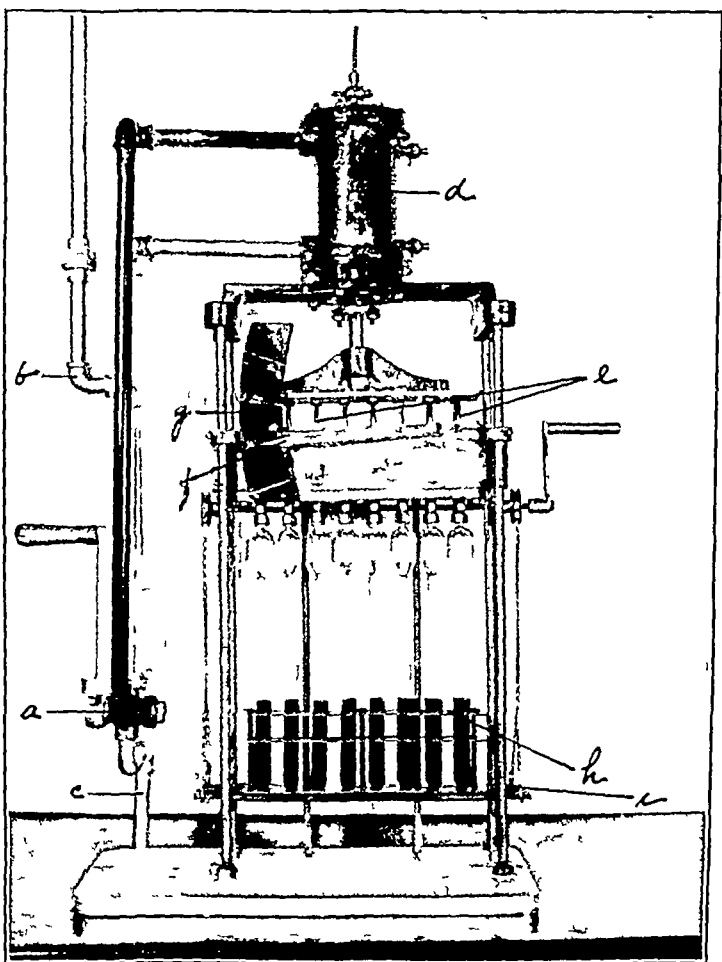


Fig 1

to the amount of sugar present. For standards we use glucose solution of 0.13, 0.2, 0.3, 0.4, and 0.5 per cent. Amounts of sugar below 0.13 per cent are considered negligible and are reported negative, those from 0.13 to 0.5 are reported directly in percentage and those above the latter figure are titrated quantitatively with Benedict's solution.

These methods have been found by us to be highly satisfactory and have done much to eliminate the personal factor of individual judgment, especially as to what terms to employ in describing the amount of albumin found. How-

ever, in order to adopt these methods we were compelled to develop apparatus which would make pipetting much less laborious and time consuming than is usually the case. The apparatus put into service in 1927 has been used continuously since that time with great success and satisfaction.

An examination of Fig. 1 gives a clear idea of the processes, two such pieces of apparatus are used, one to pipette the urine necessary for both determinations and the other to pipette the reagents. In the operation the liquids are drawn into the glass pipettes by means of suction produced in cylinders above, which originally consisted of metal syringes arranged vertically in two parallel rows. Later a solid block of brass was accurately bored and pistons fitted as in automobile engine construction. These pistons were operated by hand with a crank through a number of gears. The lost motion with this type of construction and the rather heavy effort necessary, led us to the consideration of further improvements.

In the present apparatus hydraulic pressure is used as a source of power. This is accomplished by means of a four-way valve at "a". Water is admitted through pipe "b" and discharged through pipe "c" after passing through cylinder "d". Cylinder "d" is approximately three inches in diameter and the water pressure here available exerts a force of approximately 300 pounds on the pistons, which is much more than adequate for the purpose. Even with this amount of pressure the movement of the pistons "e" can be very delicately controlled. The indicator "f" shows the amount of fluid withdrawn or discharged on the graduated scale "g". The rack "h," containing 16 tubes, is supported and placed in position by means of the elevator "i". The entire apparatus is held rigidly in position by screws through the base into the laboratory table.

#### COMMENT

Our urine sets range from 480 to 640, and these are analyzed with a degree of efficiency and accuracy never attained in former times when it was necessary to give each specimen a number of handlings. We are highly satisfied with the results and can recommend the apparatus and methods employed to such laboratories as have a sufficient volume of work to make them feasible and practical.

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## AN OPERATING TABLE FOR MICE\*†

MICHAEL LEVINE, PH D, AND VICTOR KUGEL, M D, NEW YORK CITY

IN STUDYING the effects of the thyroid upon tumor growth, the authors subjected a series of mice to thyroidectomy. It was soon found that the mortality encountered was out of proportion to the operative technique. Likewise, it was noticed, in common with others who attempted these delicate operations, that it was necessary to keep the temperature of the animal as nearly normal and constant as possible during the operation because of the apparent severe

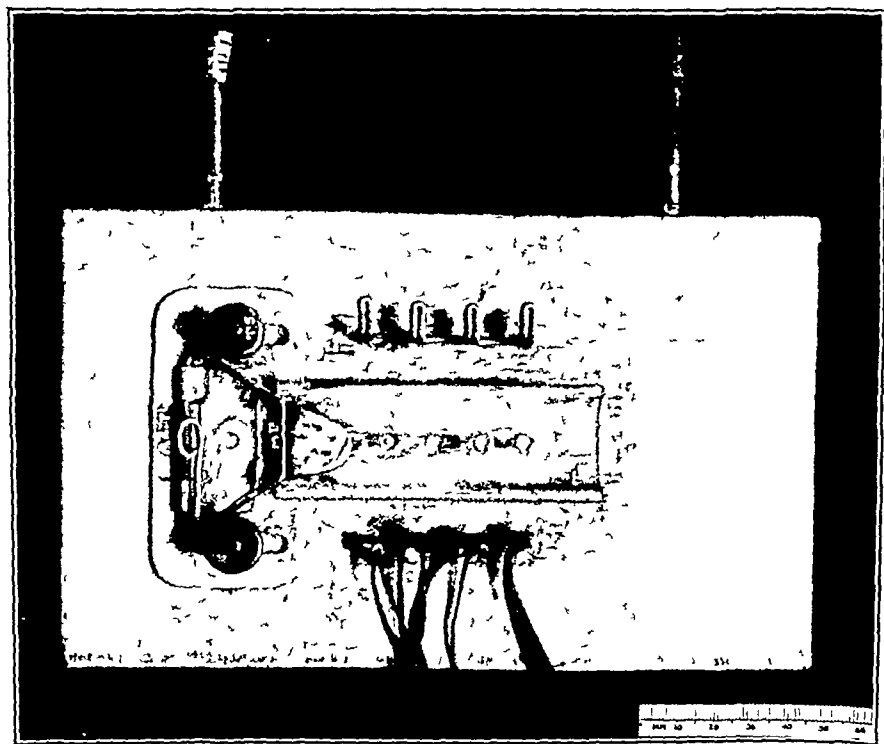


Fig 1—Surface view of operating table for mice. *I O* Inlet and outlet corked *ad pl* adjustable plate *T S*, thumbscrew *M P*, mouthpiece *Tr*, trough.

shock which follows. Owing to the size of the animal, it was also found desirable to provide means for fully exposing the operative site and to firmly immobilize the head and body without trauma.

A suitable operating table which would fulfill these needs could not be found on the market. After a considerable number of trials, with various devices, we constructed an operating table as shown in Fig 1. This table we

\*From the Laboratory Division Montefiore Hospital.  
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believe of sufficient interest because of its efficacy in maintaining the animal's warmth, its ease of construction its relatively low cost of manufacture, and because of its invaluable aid in delicate operative work on the neck organs. Through the use of this operating table we were able to reduce the mortality while thyroidectomizing mice from 50 to 60 per cent to 10 per cent and less. We generally thyroidectomized from ten to twelve animals at one sitting.

We are describing our operating board at this time because the results of our studies are not yet entirely prepared for publication, and we feel that the table may be of use to others who are operating on mice at this time.

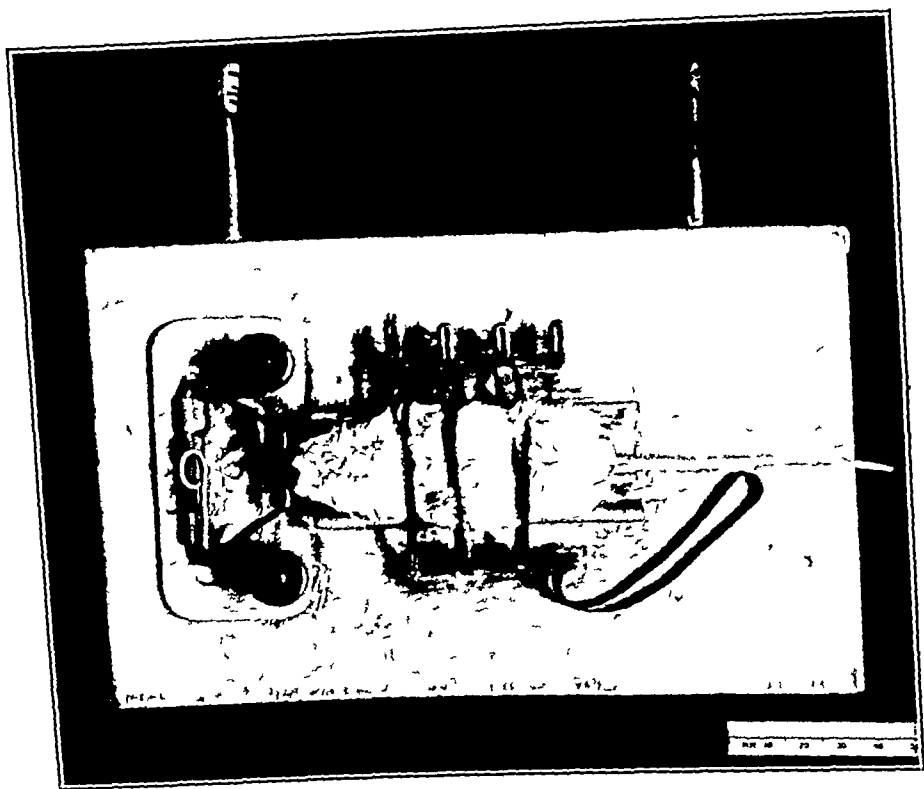


Fig 2—Mouse in position with neck well exposed.

The principle involved consists of passing water of required temperature through the operating table so as to give off heat by conduction and radiation, to and about the animal. The operating table consists of a chamber 20 cm  $\times$  11½ cm  $\times$  2½ cm made of galvanized iron sheet metal (24 gauge) with two small copper tubes soldered into one side, at about 4 cm from each end (see Fig 1). The capacity of this box is approximately 575 cc. One of the tubes serves as an inlet and is attached by a rubber hose to a spit bottle filled with water heated to the required temperature. The other tube entering the box is attached to a small rubber hose which empties into a waste receptacle. As the water in the box becomes cool the water from the spit bottle may be forced into the box until the required temperature is reached. The temperature of the water may be raised in this way between operations. The operating board inlet

may be attached to the hot water faucet of the sink, a practice commonly used by us, and a steady stream of water may pass through the chamber. When only one or two animals are thyroidectomized at one sitting, the chamber may be filled with water and the copper tubes corked.

To the broad surface of the operating table is riveted a small metal trough approximately the size of a mouse of 20 to 40 gm. The head is supported by a small cork which fits under the neck. At the cork-end of the trough there is a small metal plate which is adjustable and is made fast to the operating table by two thumb screws. On this plate is attached a hinged wire mouthpiece (see Fig. 1), held down in position by a delicate spring. The mouthpiece is so fashioned as to fit into the mouth of the animal and under the front teeth of the upper jaw. The mouthpiece, once adjusted, is kept in place by the delicate spring.

Along one side of the trough a series of eyelets are soldered to the table, and on the opposite side, an equal number of hooks. Fresh rubber bands of required elasticity are noosed in the eyelets and the animal is held firmly in the trough by stretching across the legs of the animal the rubber bands which are fastened to the hooks. The number of bands to be used depends upon the size of the animal. A rubber band stretched across the upper legs and another over the body holding down the lower legs, are usually sufficient (see Fig. 2).

After the animal is anesthetized, the body is fixed in the trough by the use of rubber bands. The wire mouthpiece is placed under the upper teeth, and the neck is stretched by moving the adjustable plate forward until the neck is made slightly taut. The thumb screws on the adjustable plate are made fast, in place, and the operation may then go on.

A more detailed description of our methods of operation and accessories used in the removal of these extremely small organs will be published in a subsequent report together with our findings.

We are indebted to Mr. Harry Hawker for the manufacture of this table.



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**SEDIMENTATION TEST, A Study in Correlation of the Sedimentation Test, Filament-Non Filament Count, and the White Cell Count in Gynecology, Yates, H. W., Davidow, D.M., Putnam, E. and Ellman, F. Amer Jour Obstet & Gyn 25 201, 1932**

From an extensive study the following conclusions are drawn

- 1 A The percentage of accuracy of white blood count in these two studies was 67.5 per cent
- B That of the filament nonfilament count was 77.2 per cent
- C The accuracy of the sedimentation rate was 91.4 per cent
- 2 There was a definite correlation of the sedimentation rate and filament nonfilament count in the infectious conditions
- 3 The white blood count was not dependable
- 4 In the cases of marked anemia the sedimentation rate was more rapid than normal
- 5 In the case of abortion with bleeding there was no correlation between the three tests

**CARBON MONOXIDE POISONING, Methylene Blue a Synergist, Not an Antidote, for, Haggard, H. W., and Greenberg, L. A. J. A. M. A. 100 2001, 1933**

From experimental studies on dogs it is concluded that there is no valid basis, theoretical, experimental or clinical, for the belief that methylene blue is an antidote for carbon monoxide asphyxia

The chief effect of methylene blue is to convert some of the hemoglobin of the blood into methemoglobin. By thus further diminishing the oxygen carrying capacity of the blood, methylene blue acts as a synergist with carbon monoxide in promoting asphyxia. It probably exerts also other deleterious effects.

Experimental evidence is here presented showing that the administration of methylene blue in carbon monoxide asphyxia may induce fatalities that would not otherwise occur. Illness attributable to the effects of methylene blue persists after recovery from carbon monoxide asphyxia.

These conclusions reinforce previous evidence that hypodermic and intravenous medication is more likely to be injurious than remedial in the treatment of carbon monoxide asphyxia.

**SPIROCHETES A New Method for the Cultivation of Spirocheta Recurrentis, Yuan Po L. Kitasato Arch Exper Med 10 78, 1933**

One cubic centimeter of sterile white of an egg is placed in a test tube and heated to 80-85° C for about thirty minutes. The white of one egg is sufficient for 15 tubes. The yolk of the egg is placed in a flask containing 400 cc of sterile 0.8 per cent saline and thoroughly shaken. The contents of the flask are then allowed to settle for some days in the ice chest. The  $P_{H}$  of the supernatant fluid is about 7.0 and it keeps its properties for at least two months. About 5 cc of this liquid is added to each tube of heated egg white and the resulting medium either used at once or first heated to 55° C for half an hour. The eggs must be fresh and those whose shells are a dark biscuit color produce the most favorable culture medium.

One or two drops of citrated infected blood are added to each tube and the medium then covered with sterile paraffin oil and kept at 35 to 36.5° C. Subcultures are made every three to five days by transplanting about 0 to 5 cc of a good culture together with a small drop of citrated human or rabbit blood, then covering with paraffin and incubating. The citrated blood is prepared by mixing 2 cc of blood with 0.2 cc of a 10 per cent sodium citrate solution also containing 10 per cent glucose. The citrated blood keeps for at least three weeks if kept in the ice chest.

#### GASTRIC ANALYSIS Histamine Test Meals, Pollard, W S Arch Int Med 51 903, 1933

An analysis has been made of 988 consecutive histamine test meals. Six hundred and eighty-four of the patients showed no evidence of disease, and standards of normal for gastric acidity and volume of secretion were derived from these data.

The mean total acidity for male subjects ranged from 101.1 units at the age of 25 to 67.1 units at the age of 65. The mean total acidity for female subjects ranged from 82.2 units at the age of 25 to 66.7 units at the age of 65. This included normal subjects who had anacidity. In the case of the male subjects, there was a definite correlation between age and acidity.

The mean maximum ten minute volume of secretion for male subjects ranged from 39.7 cc at the age of 25 to 24.9 cc at the age of 65. The mean volume of secretion for the female subjects ranged from 33.1 cc at the age of 25 to 21.7 cc at the age of 65. There was a definite correlation between age and volume in the two sexes.

In both sexes the total gastric secretion declined with age at about the same rate.

There was a steady increase in the incidence of anacidity from youth to old age, and at all age periods up to 60 the incidence was higher in female than in male subjects. The incidence for the normal male subjects was 10.7, and for the normal female subjects, 14.1 per cent.

In 130 cases of duodenal ulcer, 91.3 per cent of the subjects had a total acidity and 79.2 per cent had volumes of secretion higher than the mean values of normal persons of the same age and sex.

In 36 cases of gastric ulcer, 91.7 per cent of the patients had a total acidity and 75 per cent had volumes of secretion higher than the mean values of normal people of the same age and sex.

In 56 cases of carcinoma of the stomach, the incidence of anacidity was 69.6 per cent. In the 56 cases, there was only 1 patient who had anacidity and 3 who had volumes above the mean normal value for the same age and sex.

Eighty-seven and one-tenth per cent of male patients with gastric ulcer and 92.5 per cent of male patients with duodenal ulcer had total secretions above the normal mean for age, and 100 per cent of male patients with carcinomas had total secretions below this mean.

The diagnostic value of the histamine test meal in differentiating benign from malignant lesions of the stomach is discussed.

In a miscellaneous group of cases, no evidence could be found that any particular disease, except pernicious anemia, was associated with a characteristic type of gastric secretion.

#### LIVER FUNCTION, Clinical Evaluation of Galactose Tolerance Test, Banks, B M, Sprague, P H, and Snell, A M J A M A 100 1987, 1937

The authors feel that the galactose test does not uniformly distinguish between obstructive and toxic or infectious types of jaundice, although it may furnish valuable corroborative data in doubtful cases. In both intrahepatic and obstructive types of jaundice, strongly positive tests (excretion of 6 gm or more) should be seriously regarded and, if excretion is consistently high, are probably indicative of serious injury to the hepatic structure. The borderline group of positive tests, with excretion of only slightly more than 3 gm of reducing substance, should not be regarded as conclusive evidence against the presence of mechanical

biliary obstruction The value of the test to the individual practitioner will depend on how carefully its results are weighed against time honored clinical data and previous clinical experience with jaundiced patients

**BLOOD CHOLESTEROL** Chemical Studies in the Epileptic Syndrome, Hopkins, H  
J Nerv & Mental Dis 77 601, 1933

The cholesterol content of the blood in patients presenting the epileptic syndrome appears to have some significance in indicating the direction in which the physicochemical changes are taking place

The average whole blood cholesterol values are slightly lower in a group of epileptic patients than in a corresponding normal group

The range of variation in whole blood cholesterol values from hour to hour throughout 24 hour periods is greater in epileptic patients than in normal individuals

One case undergoing convulsive activity during the twenty four hour period of study shows a preapexymal fall in the whole blood cholesterol amounting to 11 per cent of the average cholesterol value for the day

**UNDULANT FEVER** Brucella (Alkaligenes) Infections in Man The Intradermal Reaction as an Aid in Diagnosis, Yeckel, H. C, and Chapman, O D J A M A 100 1885, 1933

The following conclusions are drawn from this study

The intradermal reaction is a definite aid in the diagnosis of brucella infection in man

The test is harmless and does not disturb the patient

Negative agglutination and intradermal reactions definitely exclude clinical undulant fever

The skin remains sensitive to the specific antigen long after the active infection

In the presence of a negative agglutination reaction, the intradermal reaction may be positive and thus lead to a definite diagnosis

Brucella abortus 80 is a good antigen to use for the intradermal test It is not necessary to make a series of tests using two or more antigens

For proper interpretation, the result must be read at approximately the ninety sixth hour

The nonspecific reactions usually appear within the first twenty four or forty eight hours and disappear by the seventy second or ninety sixth

A positive reaction may mean a former infection

**BACTERIOPHAGE**, Studies on Cholera, Technic, Asheshov, I N, Asheshov, I, Khan, S, and Lahiri, M N Ind J M Research 20 1101, 1933

This paper, which does not lend itself to abstraction, should be read with interest and profit by all who are engaged in work with bacteriophage because of its wealth of technical detail based upon the extensive practical experience of the authors

The following precautions are emphasized as essential

1 Prevent bacteriophage from being spread all over the laboratory, treating each filtrate as a highly contagious liquid If spilt, use freely some chlorine solution, electrolytic chlorine (E C) for instance This solution must be in hand's reach of every worker Hands must be frequently washed in it

2 Have a piece of cotton wool moistened in E C on a Petri dish cover in front of you when transferring or plating bacteriophage Drain the loop on it before flaming the droplets of the liquid brought suddenly into the flame burst and scatter bacteriophage all around before it is killed in the flame

3 When pouring out bacteriophage suspensions from any container (e.g, from a test tube into the funnel), flame the rim of it not only before but after pouring out as well The container before sterilization is likely to be handled by attendants, who will soil their hands and spread bacteriophage all over the laboratory

4 Flame the rim of the filter candle before inserting the stopper of the funnel. When disconnecting filter candles, wipe the place of connection with cotton wool moistened in E C before touching the candle.

**LIVER FUNCTION, Clinical Value of the Rose Bengal Test for the Determination of the Total Functional Capacity of the Liver, Rao, M V R. Ind J M Research 20 1009, 1933**

As a result of this study the following conclusions are advanced:

1 Rose bengal (diiod tetra chlor fluorescein), a dye belonging to the triphenylmethane series, has been utilized to study the chromogenic function of the liver for determining the total functional capacity of the organ.

2 The modified technique described by Delprat and Stowe (1931) was adopted in this investigation and the test is described in detail.

3 The dye is entirely nontoxic to the human tissues and no untoward symptoms were seen in any of the cases after intravenous injection of the dye in doses of 100 mg. for the adult.

4 Marked retention of the dye was found in cases of cirrhosis and malignant disease of the liver. In cases of metastatic malignancy of the liver the excretion of the dye depends upon the amount of functionally active liver tissue still remaining. Marked retention of the dye without bilirubinemia constitutes an important observation in cases of decompensated portal cirrhosis of the liver. The causes of this marked retention of the dye in portal cirrhosis are discussed and it is shown that the secondary anemia, which is seen in the later stages of the disease, cannot account for this marked retention.

5 Jaundice associated with toxemias of the liver always produced retention of the dye.

6 Slight retention of the dye was seen in long standing cases of passive congestion of the liver resulting from cardiac decompensation and in cases of inkylotomiasis, in which the secondary anemia had persisted for a long time.

7 The test is a valuable aid in differentiating ascites due to cirrhosis of the liver from other conditions producing ascites, e.g., cardiac decompensation, nephritis, or peritonitis—simple, tubercular or malignant.

8 The results obtained in this investigation are in agreement with those obtained by the originators of this test. The test is more likely to be positive in those conditions in which there is diffuse destruction of the hepatic parenchyma, than in those with localized affection. The test does not show any specific type of organ damage, but only denotes the amount of functionally active liver tissue still present.

9 It is pointed out that, while the retention of the dye is of great significance in the diagnosis of cirrhosis of the liver, its value in the prognosis is very limited. It is suggested that no useful purpose is served in calculating the "total function" of the liver, from the percentage of dye present in the blood at the end of eight minutes, by the formula given by Delprat and Stowe (1931).

10 Taken in conjunction with the clinical observations and the results of other tests, the rose bengal test constitutes a valuable aid in the study of hepatic diseases.

**VARIOLA, Complement Fixation In, Venkataraman, K V. Ind J M Research 20 4, 1063, 1933**

The author corroborates the presence of complement fixing antibodies in smallpox and reports that they are regularly present and can be satisfactorily demonstrated using a calf lymph antigen.

The vaccine was diluted 1:40 with normal saline and, when the coarser particles settled, the supernatant fluid was used for antigen and found not anticomplementary in double this strength.

**FILARIASIS, Complement Fixation In** Lloyd, R. B., and Chandra, S. N. Ind J M  
Research 20 1197, 1951.

A series of eighty nine cases of various clinical types of infection with *W. bancrofti* was examined by the complement fixation reaction, using as antigen extracts, variously prepared of *D. immitis*. Twenty three positive reactions were obtained. A series of fifteen cases of infection by other helminths was also similarly examined, yielding three positive reactions, all of which were in guinea worm disease.

It is shown that cases of acute or subacute lymphangitis tend to exhibit two distinct types of immunity response. In one type the finding is a positive complement fixation reaction associated with an eosinophilia, the total white count and polymorphonuclear percentage being within normal limits. In the other type the finding is a negative complement fixation reaction with a polymorphonuclear leukocytosis. The authors conclude, accordingly, that there are two distinct types of attack in such cases which they designate the toxic and septic types respectively.

Repeated examination by the complement fixation test of cases of acute or subacute lymphangitis with a positive complement fixation reaction has demonstrated that as the attack passes off the reaction becomes negative.

The positive complement fixation reaction in whatever type of filarial lesion it may be found, is always associated with an eosinophilia, and, so far as we can see at present, the disappearance of the positive complement fixation reaction does not seem to affect the eosinophilia, though this point requires further examination.

As a result of various considerations, the authors have suggested that the preliminary immunity response in infection by *W. bancrofti* is anaphylaxis plus an eosinophilia, and that in circumstances favorable to large releases of filarial toxin the positive complement fixation reaction is superadded as a further stage of immunization, the clinical result of the increased toxin releases being an "attack" in types of obstruction where the toxin releases are normally small, such as in blockage of the limb lymphatics, but no attack in circumstances where the lymphatics are still freely open as in chyluria.

Two cases are referred to showing the effect upon the positive complement fixation reaction of surgical treatment of the antigenic factor.

Of four cases of guinea worm disease three reacted positively and the positive reaction remained unchanged for long periods. The long duration of the positive reaction is probably due to the absence of lymphatic obstruction, bringing the findings in guinea worm disease into line with Fairley's findings in *L. loa* and the author's findings in chyluria. As in chyluria, eosinophilia was present in the one case of guinea worm disease which yielded a negative complement fixation reaction as well as in those which reacted positively. Adequate facilities for the study of guinea worm disease are not available in Calcutta, but it is suggested that examination of this test will probably not only lead to a valuable means of diagnosis of guinea worm disease, but will also provide important immunological data which may be applicable to the more complex cases of infection with *W. bancrofti*.

From Fairley's results in *L. loa* and the author's in guinea worm disease it seems likely that the complement fixation test by means of *Dirofilaria* extracts is a group reaction for the worms of the filarial family generally.

As the clinical manifestations of *W. bancrofti* infection are largely those of lymphatic obstruction whereby the antigenic factor is to a progressive extent cut off from the circulation, no serological test could be expected to diagnose all types and stages of the disease, but in a somewhat restricted field the test seems likely to be useful in diagnosis.

So far as examined, the specificity of the test seems quite satisfactory. Apart from guinea worm disease, no positive reactions were obtained in other helminthic diseases except in two cases of hookworm infection, in both of which *W. bancrofti* infection was also present.

As there is no method at present of combating the filarial toxin directly the demonstration that some attacks of lymphangitis are of purely toxic origin is not of immediate therapeutic value, except in so far as it suggests that probably no benefit is to be looked for from vaccine treatment in this type of attack.

**TUBERCULOSIS** Acid-Fast Bacilli in the Stomach Lavage and Feces of Tuberculous Children, Kereszturi, C, Hauptmann, D, Schick, B, and Mishulow, L J A M A 100 1481, 1933

Among 101 tuberculous children, only 27.7 per cent showed tubercle bacilli in the stomach content

The ages of the children ranged from eight months to sixteen years

The type of the cases included in the study varied from children who had no other pathologic clinical signs than a positive tuberculin test to children who had bilateral destructive pulmonary tuberculosis

If guinea pigs were not used, the smear examination of stools yielded 7 per cent less positive results than the more cumbersome stomach lavage

The Armand Dehille modification did not, in the author's hands, improve the routine stomach lavage first suggested by Meunier

Sputum and throat swab examinations gave positive results less frequently than did stool or stomach lavage examinations

Clinically, destructive pulmonary tuberculosis cases showed in 75 per cent positive stomach lavage results against 28 per cent among the clinical nondestructive pulmonary tuberculosis cases. The other forms of tuberculosis cases did not show positive results except one bone tuberculosis case

Younger children did not show positive results more often than older ones

Clinically, inactive cases yielded only negative results, slightly and very active cases showed different proportions of positive results, and activity seemed to parallel the frequency of positive examinations

Positive stomach lavage for tubercle bacilli had severe prognostic significance

Pathologic chest signs, fever and cough generally parallel the frequency of positive results

The average age of the patients with positive stomach lavage was two years higher, the malnutrition somewhat less and the average gain of weight about half as fast as among the cases who gave negative stomach lavage results

As far as sputum and feces examinations were concerned, the routine hospital examination yielded as good results as the careful research study, because more frequent examinations were made

**GASTRIC JUICE**, Protein and Non-Protein Nitrogen Determinations on, Martin, L J A M A 100 1475, 1933

In the normal gastric juice, protein nitrogen and nonprotein nitrogen fractions (amino acid, urea, uric acid and ammonia) were found in amounts that varied within but small limits

In the benign achlorhydrias the amounts were found to be increased approximately twofold

In the cases of pernicious anemia the same increase was found and associated with still greater amounts of amino acid and urea

In carcinoma of the stomach associated with achlorhydria, large amounts of protein and nonprotein nitrogen were found

**PNEUMONIA**, Blood Picture in, Rosenthal, N, and Sutro, C J Am J Clin Path 3 181, 1933

In addition to noting ordinary blood changes, observations on pathologic cytoplasmic alterations (toxic granules) are a valuable aid in the diagnosis and prognosis of cases of pneumonia

Pathologic cytoplasmic alterations (toxic granules) are present in all cases of lobar pneumonia, are diminished in bronchopneumonia, and usually are absent in conditions simulating pneumonia

In recovered cases, the degenerative index, that is the percentage of cells showing toxic changes, rises rapidly (not infrequently to 100) either prior to, or immediately after, the crisis or fourth week.

The degenerative index is lower in alcoholic patients suffering from pneumonia.

In fatal pneumonia the degenerative index is frequently 100, its persistence at that level presages a fatal prognosis.

**PNEUMOCOCCUS TYPING, Immediate Directly from Sputum by the Neufeld Reaction,**  
Sabin, A. B. J. A. M. A. 100 1584, 1933

Two small flecks of sputum are placed on a cover slip (about 22 by 50 mm) with a platinum loop, the diameter of which preferably does not exceed 2 cm. When the sputum is very tenacious, as it frequently is, it may be necessary to use another wire for getting the sputum off the loop. To each bit of sputum an equal quantity of the undiluted rabbit serum (Type I serum to one, and Type II serum to the other) and a loopful of standard alkaline methylene blue was added. A special deep, large, hollow ground glass slide, big enough to cover both drops, was used, this special slide is convenient but not essential. The edges of the slide are smeared with petrolatum, it is placed over the cover slip, and the preparation is inverted. The examination is made with the oil immersion lens and an artificial blue light. Although the reaction occurs almost immediately, it is best to delay the examination for about two minutes to allow for proper diffusion of the serum.

It is important to observe that ordinarily, in the hanging drop preparation, pneumococci in sputum show no capsules, occasionally a faint halo of light but without any definite outline may be seen about the organism.

However, in the type specific mixtures of sputum and serum one finds the pneumococci surrounded by peripheral zones of characteristic appearance and distinct outline. This peripheral zone consists of a refractile substance which does not take the stain and which may be described as having a ground glass appearance, the organism within it is stained blue. The size of this zone of "quellung" varies in different sputums with organisms of the same type, generally, Type II pneumococci presented the larger zone. It is important to stress, however, that it is not so much the size of the zone as its characteristic appearance which determines a positive reaction. It is also necessary to state that this reaction does not depend on agglutination of the pneumococci, although it occasionally occurs in the sputum, but on the appearance of the individual organisms, furthermore, this appearance is so characteristic that the finding of even a single diplococcus which shows it is sufficient to diagnose the type.

**TUBERCULOSIS, Identification of Bovine Type from Sputum,** Girdwood, R. O. J. Path & Bact. (Edinburgh) 36 153, 1933

A morning specimen of sputum is collected and an equal quantity of one normal sodium hydroxide solution is mixed with it for thirty seconds and centrifugated at 2,000 revolutions per minute for twenty minutes. If the specimen is more than usually viscous, the mixture is incubated at 37° C for twenty minutes before centrifugating. The supernatant fluid is decanted, the deposit is then roughly neutralized with three or four drops of normal hydrochloric acid solution, mixed, and seeded on four slopes of egg medium. The tubes are incubated, examined weekly and discarded in three months if no growth is obtained. In a few instances in which the sputum is "positive" on microscopic examination and yet no growth is present or in which, although microscopic and cultural examinations are negative, the clinical evidence strongly suggests that tubercle bacilli may be present in small numbers, the reverse method of concentration is employed, hydrochloric acid being used as the concentrating reagent and sodium hydroxide for neutralization. From the 384 cases of pulmonary tuberculosis, twenty-two bovine strains were isolated. In any given case of pulmonary tuberculosis, the presence of tubercle bacilli of the bovine type seems to be of definitely unfavorable prognostic significance.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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### The Biochemistry of Medicine

THE relation of biochemistry to the recognition, management, and control of disease has been the subject of intensive study and, within comparatively recent years, has made a tremendous advance.

So rapid have been the developments in this field that the physician has been hard put to keep pace with them and so crowded is the medical curriculum that the medical student of today is not always as familiar with them as he should, and indeed must, be.

It is to this audience that this book is addressed, its purpose being to coordinate pure biochemistry with those applications to medicine which, after all, constitute the important phases of biochemistry for the physician.

The style is clear, simple, and easy to read and the angle of view essentially practical, and it is to be expected that the book will find a wide field of usefulness. It can be heartily recommended as an eminently practical and useful text for both student and physician.

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### Lymphatics, Lymph, and Tissue Fluid

REALIZATION of the extent and complexity of the lymphatic system is the product of innumerable investigations starting from the demonstration of the mesenteric lacteal vessels by Asellus in 1627.

Investigations of the physiology of the lymphatic system, however, have not kept pace with anatomical studies so that the exact nature of its functions and the mechanism whereby they are carried out still remains to no small extent a problem awaiting solution to which the present volume makes a distinct and valuable contribution.

Starting with a review and discussion of the structural basis of the lymphatic system, the authors next consider the entrance into the lymphatics of colloidal solutions and foreign particles, reviewing the work of previous investigators and citing their own.

The permeability of the blood capillaries and their normal limits of permeability to colloids are next reviewed following which the flow of lymph, its composition, and the tissue fluid are discussed in the light of available experimental evidence.

The final chapter of the book is devoted to the practical consideration and application of the data resulting from the experimental studies previously reviewed.

Among these are the relation of the lymphatics and lymph flow to circulatory edema and in hypertension, the effect of lowered plasma proteins and of intravenous injections, the effect of heat and light, of asphyxia and carbon dioxide inhalation, the lymph flow in anaphylactic shock and in inflammation, and finally, a discussion of attempts to prevent the entrance of lymph into the circulation and of measures designed to promote the flow of lymph.

\*The Biochemistry of Medicine By A. T. Cameron D.Sc. Professor of Biochemistry, University of Manitoba, and C. R. Gilmour M.D. Professor of Medicine and Clinical Medicine University of Manitoba. Cloth 506 pages. Baltimore: William Wood and Co.

†Lymphatics, Lymph and Tissue Fluid By Cecil K. Drinker M.D. Professor of Physiology Harvard School of Public Health and Madeleine E. Field Ph.D. Instructor in Physiology Harvard School of Public Health. Cloth 254 pages. Baltimore: Md. Williams and Wilkins Co.



A bibliography of 37 pages completes the book

The volume may be regarded as a valuable, useful, and authoritative contribution to the subject especially as the authors, in preference to merely summarizing the literature, have not hesitated to state clearly what they themselves think in relation to matters of dispute

Their object has been to give a working idea of what the lymphatics are doing in the mammalian body and to this end they present data drawn from anatomy, physiology, pathology, and immunology

That they approach the subject well qualified for the task requires no emphasis That they have achieved the end in view the volume bears witness

### Minor Maladies and Their Treatment\*

THE scope, character, and *raison d'être* of this book are well indicated by the following excerpt from the Preface

"When I was first qualified I went into general practice and soon found that though moderately well equipped in the diagnosis and treatment of diseases which I seldom encountered, I was disconcertingly ignorant in those matters about which I was most frequently consulted Pneumonia I knew and rheumatic fever and typhoid, I was so well acquainted with phthisis that I confidently recognized it in every trivial cough, and so well versed was I in heart murmurs that I was prepared to discover and treat them, with a combination of digitals and words of serious warning, even when they had no serious significance

"With knowledge concerning tabes, tumors, and trematodes I was full to overflowing, but I soon realized that I knew very little about a common cold, less about ordinary indigestion, and nothing at all about the rheumatic conditions In this dilemma I searched for a book that would lighten my darkness, but I found it not "

There are few, indeed, in whom this frank confession will not strike a responsive chord for few will debate that it is not in large measure as true now as it was when first written

The small volume does not pretend to be encyclopedic but the table of contents which follows indicates their practical nature Colds, Coughs and Sore Throats, Indigestion, Constipation, Diarrhea, Vomiting and Giddiness, Rheumatism, Neuralgia and Headache, Salient Symptoms, Minor Glandular Insufficiencies, General Health, Advancing Years, Insanity, Some Drugs and Their Uses

Even the most cursory glance through the book suffices to make obvious its eminently practical trend and that the methods and advice given are based upon a wide and extensive experience The style is simple and not only catches but holds interest, enlivened by touches of humor and permeated throughout with common sense, which readily explains the passage of this book through six editions and warrants the presumption that there will be many more

### Gastric Anacidity Its Relations to Disease†

THIS, the latest of the Macmillan Medical Monographs, presents an authoritative and comprehensive survey of a subject which, while of definite clinical importance, has long been fogged by the perpetuation of erroneous statements upon which continued repetition has conferred a fallacious factual status

The authors begin their work with a definition, accepting for clinical discussion the concept long based upon the reaction to amino azobenzol, and then proceed to a discussion of methods for the determination of gastric acidity They emphasize, however, that as it is clinically used the term "anacidity" signifies only that a yellow color is obtained with

\*Minor Maladies and Their Treatment. By Leonard Williams M.D. Cloth ed 6 pages 120 William Wood & Co Baltimore Md

†Gastric Anacidity Its Relations to Disease By Arthur L Bloomfield M.D Professor of Medicine Stanford University, and W Scott Pollard M.D Instructor in Medicine Stanford University Cloth 188 pages 22 figures 12 tables The Macmillan Co New York

Topfer's indicator and that true anaclidity exists only when uncontaminated gastric juice secured after histamine stimulation presents a neutral reaction ( $P_{H}$  7.0) by hydrogen ion determination

The various test meals and their deficiencies are well discussed, the authors recommending the histamine test and describing their technique in detail

A chapter on the history and classification of acidity reveals the confusion surrounding this subject. The authors' classification follows:

"I True Anacidity A No reaction to dimethyl, even after histamine stimulation, usually, if not always anatomical lesions B No reaction to dimethyl but slight acid excretion after histamine

"II False Anacidity A Relative No reaction to dimethyl after usual test meals but definite acid production after histamine, anatomical lesions often absent B Transient Temporary abeyance of secretion not dependent upon anatomical lesions but upon some other demonstrable cause C Basal No free acid production under basal conditions but free response after stimulus (test meal or histamine), lesions not necessarily present"

True anacidity is next discussed in detail and, while occurring almost invariably in pernicious anemia, and with great frequency in gastric carcinoma, the authors emphasize its occurrence in the absence of any definite disease

This "unexplained acidity" is considered at some length but remains, as yet, an unexplained problem

Following a discussion of the etiology and clinical features of true acidity, false acidity is considered, then the acidity of pernicious anemia, and that encountered in gastric carcinoma. The association of acidity with other conditions is illustrated by a comprehensive table (21 pages). The prognosis and therapy of acidity and a final summary complete the book

In the last analysis, despite the extent of the investigations which have been recorded the established facts concerning acidity may be very briefly summarized

It is neither a disease nor a definite entity but a functional state which may be temporary or permanent, which may or may not be accompanied by lesions of varying nature and degree, many of the more minimal of which have not been shown conclusively to be the direct cause of disturbances of secretion. That acidity is the precursor or cause of certain diseases, while a possibility, has not been finally proved. There is no special treatment

The outline given above indicates the comprehensive character of the monograph and the association of the authors with this field of investigation assures a critical and authoritative presentation of the data they have assembled

In style the book is easy to read and to understand and the format leaves little to be desired

The volume may be regarded as a distinct and useful contribution to a much discussed but little understood subject and, as such, should be welcomed by the physician who must inevitably in practice encounter many cases presenting disturbances of gastric secretion as a primary or secondary symptom

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Richmond, Va

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## EDITORIAL

### Bilirubinemia and Pneumonia

CLINICAL medicine presents few pictures more dramatic, and at the same time more inexplicable, than the sequence of events which comprise the crisis in lobar pneumonia

Just what occurs, and how it occurs, to produce the striking change in the previously desperately ill patient, who suddenly and dramatically is now seen to have "turned the corner" and taken his first step forward on the road to recovery has long been a baffling puzzle to physician and pathologist alike

In 1928 Elton,<sup>1</sup> recalling the familiar bile solubility of the pneumococcus, advanced the suggestion that in lobar pneumonia due to the pneumococcus recovery might be influenced, in part at least, by lysis of the pneumococci by the bile present in the circulating blood and, as a corollary based upon this assumption, and granting it to be true, that changes in the icterus index during the acute course of lobar pneumonia might have some prognostic value

In support of this purely *a priori* hypothesis he has carried out and reported studies of sufficient interest to warrant summary and review.

Preliminary studies in seven hospitalized cases demonstrated not only that there was a definite disturbance in the icterus index during the course of lobar pneumonia but also suggested a definite relationship between the degree of bilirubinemia, as determined by the icterus index, and the acute course of the disease. The latter conclusion arose from the fact that in three out of four recovered cases there was a definite rise in the icterus index while in the fatal cases the icterus index determinations suggested either a failure of the blood-bile concentration mechanism, or an inadequate concentration early enough in the course of the disease.

From a subsequent series of thirty cases<sup>2</sup> studied during 1929, it became evident that a latent jaundice associated with toxic hepatitis occurs in primary lobar pneumonia more commonly than heretofore has been appreciated, and that while the jaundice has no apparent relation to the anatomic location of the lobe involved, it was encountered more frequently with right-sided involvement as well as, counting the two upper right lobes as one, with upper lobe consolidation. Adequate explanation for these observations is not apparent.

In this series also determinations of the icterus index were not only of definite diagnostic value, not only in the differentiation of primary from secondary lobar pneumonia but also from bronchopneumonia, but had, in addition some prognostic value in that an elevation of the icterus index occurred rather consistently in the recovered cases.

These studies have now been reported upon two hundred and twenty-four cases of lobar pneumonia in each of which daily determinations of the icterus index, direct Van den Bergh reactions, and quantitative estimations of the serum bilirubin have been correlated with results of distinct interest and definite value.

The study very soon developed numerous ramifications and, as yet, is far from complete but enough has been done to suggest the importance of its continuation and extension and to warrant consideration of the statements to follow.

In the first place Elton's studies have definitely established the fact that, whether clinically evident or not, a latent jaundice demonstrable by icterus index determinations is a constant finding in primary lobar pneumonia.

Second, the icterus index reaches its highest curve in cases terminating in a true crisis but usually subsides precipitately upon the establishment of a fluid pleural exudate, such a falling curve always being associated with a potential empyema.<sup>3, 4</sup>

Third, when the pneumonia is *pneumococcic* in origin, and not otherwise, the daily icterus index graph is of definite prognostic value as indicated by the findings in the following summary.

Approximately 30 per cent of hospitalized cases of lobar pneumonia do not form pleural effusions. In such cases the mortality ranges from 75 to 80 per cent, recovery being apparently entirely dependent upon the movements of the bile-serum concentration as shown by the icterus index.

In those cases showing the presence of fluid exudate, 70 per cent of the series, the mortality was 15 per cent

The prognostic interpretation of the icterus index depends upon (1) The maximum index obtained and, (2) the presence or absence of a fluid pleural exudate arising from each lobe involved, the index in the absence of such fluid tending to oscillate upward while in the presence of fluid the curve descends toward the normal level

A striking feature of the investigation was the marked difference influencing the interpretation of the curves encountered in the white and negro race

In the white race whenever the icterus index was within the zone up to and including 16.6 the mortality of the disease was 100 per cent. With indices not exceeding 18.7 to 30, with or without the presence of fluid exudates, there were no deaths, while cases falling within this zone but without exudate, the day of the crisis corresponded with the attainment of the maximum icterus index which, following the drop during the crisis, remained above normal for several days

In the negro race, on the contrary, recovery occurred only when the icterus index exceeded one hundred

So constantly was this difference noted that the suggestion is advanced that the phenomenon is a biologic constant and essentially negroid in character, an assumption strengthened by its occurrence in an apparently white male later admitting negro ancestry

For this phenomenon, which Elton speaks of as "the riddle of the icterus index 16.6," no adequate nor satisfactory explanation can as yet be advanced

The characteristics of this phenomenon are thus summarized by Elton

"1 As the icterus index ascends in the absence of a direct positive Van den Bergh, it is halted in its rise at 16.6 while at this point the quantitative bilirubin increases sharply to disproportionately high levels

"2 As the icterus index rises from 16.6 to 30, a definite downward loop in the quantitative bilirubin takes place, which may continue downward with the rising index, or may recover from its fall to regain at 30 approximately the value it had at 16.6

"3 In familial jaundice, pernicious anemia, and in the newborn the rise of the icterus index above 16.6 is usually accompanied by an anomalous type of direct positive Van den Bergh reaction, an immediate golden accentuation instead of the typical reddish or amber color. In lobar pneumonia, however, should this golden reaction be present, it is completely masked by the invariable presence of the reddish direct positive reaction

"4 The negro race has not as yet been found to exhibit this phenomenon which thus far appears to be associated with icterus only in the white race

"5 Icterus ascending with a direct positive Van den Bergh does not exhibit the 16.6 phenomenon in its complete form for either the halt at 16.6 is prolonged, as in many fatal cases of lobar pneumonia in the white race, or else as 16.6 is passed, no subsequent downward loop in the serum bilirubin is manifest (except in lobar pneumonia in the white race)

"The icterus of lobar pneumonia in the white race, then, exhibits the same characteristics in its changes as are exhibited in the type of icterus known as 'hemolytic' except for the fact that the Van den Bergh reaction is direct positive."

From the data thus assembled a purely hypothetical bilirubin therapy in lobar pneumonia is suggested.

It is obvious that many factors must be taken into consideration and more extensive studies undertaken before this possibility can become an actuality.

It is apparent, for example, that the aim of bilirubin therapy in the white race must be to cause the index to pass 16.6 but not to exceed 30, in the negro race, on the other hand, or in those of the white race presenting negroid reactions, an icterus index of 100 must be obtained.

The object of bilirubin therapy would be an increase or acceleration of lytic and autolytic processes but the influence of the presence or absence of fluid exudates must also be considered and regarded as of great and definite importance.

As has been commented upon above, there was an apparent definite relation between the occurrence of fluid exudate and the mortality which, in their presence in the series studied was only 15 per cent.

According to Elton the fluid mechanism is analogous to drainage and its successful operation is largely dependent upon three factors: (1) Unimpeded autolysis of pneumococci, (2) phagocytosis and the action of leucocytic enzymes, and (3) the bilirubin content of the fluid (dependent on the serum level).

The studies thus briefly outlined are of great interest and well warrant the consideration of clinician and investigator and deserve, as well, extension and corroboration.

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R A K

## CORRESPONDENCE

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*To the Editor*

In the current number of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE (18 725, April, 1933) Dr Harry Eagle in his article, "Precipitation Tests for Syphilis With Spinal Fluid" on page 728 makes the following statement

"The Wassermann reaction therefore remains the method of choice in the examination of spinal fluids. Until some method has been found of increasing the sensitivity of the precipitation tests, in fluids, the Wassermann cannot be entirely replaced by precipitation tests, no matter how great their technical simplicity, reliability or sensitivity with serum."

No mention is made in this article of the microscopic slide precipitation test for syphilis with spinal fluid which has now been compared with the Wassermann test in three hospitals (Lenox Hill Hospital, New York, Post Graduate Hospital, New York, and Mount Sinai Hospital, Cleveland) in 1631 tests, the results showing the slide tests to be more sensitive and no less specific than the routine Wassermans of the respective hospitals.

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3. Spiegel, L., Eller, J. J., and Rein, C. R. Spinal Fluid in Syphilis II. A Comparative Study of Five Hundred and Twenty-two Spinal Fluids by Complement Fixation and Microscopic Slide Precipitation Tests, Archives of Dermatology and Syphilology 26: 819, 1932.

Furthermore, as stated in the article describing the slide test with spinal fluid, the desirable sensitivity of the precipitation test with spinal fluid is achieved by the addition to the spinal fluid of a proper amount of acetic acid, by the use of a purified antigen emulsion of greater sensitivity than that used for serodiagnostic purposes, and by optimal mixture in an open slide chamber of proper size.

B. S. KLINE, M.D., Chief of Laboratory

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*To the Editor*

Dr Kline, in the foregoing letter, takes issue with my conclusion that the Wassermann test, properly performed, is more sensitive than flocculation tests in spinal fluid, and adduces as evidence the fact that in several hospitals, the Kline test has been found to yield more sensitive results. I had already seen two of the three papers to which he refers, and do not believe they invalidate my thesis in the slightest degree.

Wassermans vary enormously from laboratory to laboratory, and the bald statement that the "flocculation test was found to be more sensitive than the Wassermann reaction" has absolutely no general significance unless that technique is given in detail. The point at issue is the relative merit of the flocculation tests in comparison with the optimum Wassermann laboratory B, which may be wholly inadequate. Neither of the papers to which Dr Kline refers adequately describes the Wassermann technique used. The quantity of reagents used in proportion to spinal fluid, the duration of ice box incubation, the duration of the secondary

incubation, the number of imboceptor units, the method of preparing antigen we would like to know all these facts before deciding as to the validity of the conclusion that the Kline or any other flocculation test is more sensitive than "the" spinal fluid Wassermann reaction

In my own experience, a four hour ice box Wassermann test with an antigen containing 0.612 per cent sterols, using 0.2 cc of each of the four reagents, and 1.0 cc of spinal fluid, following the ice box incubation with  $\frac{1}{2}$  hour incubation at  $37^{\circ}$  C, and using a cell suspension sensitized with 3 units of imboceptor, is so far superior to the Kline or Eagle flocculation tests as to make the Wassermann mandatory. Until the Kline or any other spinal fluid flocculation technique has been compared with a Wassermann of approximately the same sensitivity as that described in my paper, and found to yield more sensitive and equally reliable results, the method of choice in examining the spinal fluid remains the Wassermann. Certainly, the burden of proof rests upon those who would discard it.

This entire discussion illustrates the necessity for a competitive serologic conference in this country, as the first step toward a much needed standardization of laboratory procedure.

HARRY EAGLE, M.D.

Harvard Medical School

#### *To the Editor:*

Dr Eagle states "that the question at issue is not flocculation test vs a Wassermann reaction, but flocculation test vs the best Wassermann available." The letter then gives details of a Wassermann test Dr Eagle considers the best and concludes that since the articles referring to the comparison of slide test and Wassermann fail to describe the Wassermann technique used, any general conclusion as to the relative sensitivity of the slide and Wassermann tests are certainly not justified.

I should like to say in reference to this reply that it makes no comment concerning Dr Eagle's failure to note or mention the method "of increasing the sensitivity of precipitation tests in fluids," contained in the first article on the Slide Test with Spinal Fluid (see reference 1, letter of May twelfth) and described in the previous letter.

Furthermore, sufficient descriptions of the Wassermann methods employed at the Lenox Hill Hospital and Post Graduate Hospital, New York, are given in references 2 and 3, letter of May twelfth, to make it clear that the pathologists at the respective hospitals were justified in employing them routinely. The Wassermann method used at Mount Sinai Hospital, Cleveland, was the Cleveland method (Am J Syph 9:765, 1925) which we have found thoroughly satisfactory and since we employed the same antigen as used in the slide test we felt that the test for test comparison was an even more conclusive one than in the usual study.

In fairness to these three Wassermann methods and many others, Dr Eagle should quote the results of a comparison of his methods with them before he admits his to be the "best available."

B. S. KLINE, M.D., Chief of Laboratory



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## *CLINICAL AND EXPERIMENTAL*

### *INSULIN RESISTANCE AND SENSITIVITY\**

W G KARR, PH D, C W SCULL, PH D, AND O H PETTY,† MD  
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THE use of insulin in the treatment of diabetes is usually accompanied with little discomfort. However, there occur cases in which there is a more or less reaction to the insulin injected. It is usually of an immunologic nature. Immunologic reactions to insulin may be divided into two classes, one, in which the reaction is local and usually accompanied by swelling, redness, and pain, and the other, in which the reaction is general, and the usual response is urticaria. Urticarial responses were more common with the very crude insulin that was used during the first period of insulin therapy. They were often attributed to the protein content of the insulin. However, with purified insulin, reactions still occur with more or less frequency.

Allan<sup>1</sup> states that the incidence of these local reactions has increased during the past five years. During the year 1930 it occurred in over 13 per cent of the cases at The Mayo Clinic. This reaction was usually mild and was relieved either by change of the type of insulin or passed off spontaneously, as the treatment continued. However, in certain cases the allergic manifestations were more severe and interfered with treatment. Usually these sensitive cases were not accompanied by resistance to insulin, but there were also certain resistant cases, which were not insulin sensitive. These mild local reactions are not uncommon in other clinics, and there is an opinion that they may occur less frequently with insulin prepared from beef than from pig.

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Received for publication October 10 1932.

†Deceased.

The following case differs from those previously reported, in that the patient was both very sensitive and very refractory to insulin. Also, the refractory condition disappeared suddenly after twenty one months and followed immediately the intramuscular injection of some rabbit serum, the nature of which will be described later. The sensitivity to insulin, however, persisted for a much longer period of time and is present, to some extent, after a period of over three years, although the patient has been without insulin therapy for a period of nineteen months.

Similar local reactions have been described in the literature. Glasberg, Somogyi, and Taussig<sup>2</sup> in discussing an insulin case, described the local reaction as follows: "From five to seven hours after the administration of insulin a large area of induration appeared at the site of injection, reaching a diameter of 10 cm. in from twelve to fifteen hours, the area was hot, angry and tender for twelve hours more and then gradually faded. The reaction was identical with several brands of insulin."

The patient of the above authors is very interesting, in that she showed a spontaneous drop in insulin requirements. This is pertinent in relation to the present case. Because of hypoglycemia, the amount of insulin was reduced from 320 units daily to 100 units over a period of six days. Dr. Taussig<sup>3</sup> has kindly given me further information since their report was made. During 1928, two years after the above described reactions, the patient was clinically well on 100 U. daily. In 1929 patient was taking about 70 U. daily. March, 1931, the patient reported that she was without glycosuria on 50 U. of insulin per day. This case differs markedly from the present case in that the refractory period was only of about three months' duration. Also, although there was a rather spontaneous recovery from the refractory condition, still the patient, five years later, is requiring 50 U. of insulin daily. Although these authors examined the serum for precipitins, no reaction could be obtained in any dilutions.

Lawrence<sup>4</sup> in discussing local insulin reactions under his group classed as "delayed reactions" describes them as follows: "In such cases during the first day or two of the injections only the usual stinging results. About the third to fifth day, hot, brawny swellings begin to appear at the site of injection, develop fully in six to twelve hours, and have usually disappeared in twelve to thirty-six hours. They may cause much pain and irritation, and can be partly alleviated by cooling lotions." He states that he has never seen them cause trouble for more than three months. He states that sometimes a change of insulin makes them reappear for a week or two.

Tuft<sup>5</sup> in a paper on insulin sensitiveness, reports a case with a marked generalized urticarial reaction. This patient was sensitive to various brands of insulin tried, and also to purified crystalline insulin. This case gave a marked skin reaction, a positive Prausnitz and Kustner reaction, and a precipitin reaction which remained positive for a short time after the generalized reaction occurred.

This patient apparently was definitely sensitive to purified insulin, but the response was generalized rather than local, as in the present case.

The patient considered in this paper showed, at all times, a marked local reaction at the site of insulin injection. The patient said it came from the very start of injections. It was first noted on the nurse's record ten days later. Soon after injection the patient noted a feeling of warmth in the area. From one to three hours later there appeared a diffuse swelling, very red, which felt much warmer than the remainder of the skin. Pain developed in the area, which persisted for twenty four hours or longer. This reaction came with every insulin injection and persisted throughout the course of insulin therapy. Later the insulin was injected at various sites over the body in order not to repeat the reaction in the same area too often. In the latter few months of insulin therapy, the local reaction changed in some respects. Instead of the diffuse swelling extending for 10 to 15 cm it became more localized and somewhat indurated. The redness did not appear, instead, a slightly elevated blanching of the skin, a rather typical wheal reaction, extending with a diameter of 4 to 6 cm about the site of injection. The patient often noted a flushing, or general feeling of warmth all over the body. The pain was present as before. No urticaria was noted until the latter part of March, 1930, when, because of the extremely high insulin requirement, some was given intravenously. When given ten units three times a day there was some urticaria, especially of the lower limbs. This was increased to a 25 U dosage and after one of these there was a sudden generalized urticaria which was relieved by adrenalin. Reducing this to 15 units three times a day there was little or no reaction noted over a period of time. As mentioned above this intravenous insulin accompanied a larger dose subcutaneously. Later when the insulin requirement dropped the intravenous insulin was stopped. After this at various times there was some urticaria after the regular subcutaneous injections. At one particular time when 90 units were given instead of the customary 45, there was a sudden marked generalized urticaria which was relieved by adrenalin.

Root<sup>6</sup> in an excellent review has summarized some thirteen conditions which might influence the resistance of the patient to insulin. Throughout the course of this patient's stay in the hospital various clinical conditions were looked for, which might, in some way, aid in explaining the refractory reaction to insulin. Various liver functional tests were instituted, but they were all negative for any hepatic dysfunction. Basal metabolic rate determinations done during August, 1929, gave readings of about -24. There was no evidence of hyperthyroidism, however, and later basal metabolic rates were normal. Outside of a few common colds and an abscess of the thigh there were no known infections present. There was a cardiac condition, but it was not accompanied by either circulatory failure or edema, which might affect the absorption of the insulin.

Of the theories advanced for insulin resistance, it would seem that only the one concerned with an enzyme of normal muscle which reacts with glucose only in the presence of insulin might be applicable. It is interesting to speculate concerning the relationship between the sensitivity to insulin and the refractory condition shown by the patient. Insulin sensitive cases are somewhat common. True insulin refractory cases are rather rare. It is noted in the

case of Glasberg, Somogyi, and Taussig<sup>2</sup> that while their case was both refractory and sensitive they did not parallel each other. In the present case sensitivity has been present throughout the refractory period and also although the patient has been without insulin for a number of months, it is still present. With the development of general allergic symptoms, and with more marked skin reactions, it would seem that the patient became even more sensitive to insulin in the later course of insulin therapy than she was in the middle of the therapy when the tests were first instituted. At present, as discussed later, the patient has lost much of the sensitivity.

The following is a summary of the Clinical Course of the patient.

Patient C. E. was a white woman, aged fifty-eight. She was admitted to the dermatologic wards of the Philadelphia General Hospital because of pediculosis corporis and a shallow varicose ulcer over the middle of the right tibia. In addition she had moderate edema of both ankles which she had first noted about four months previously. Also she had been dyspneic on exertion for about six months previously.

Her past history revealed that she had been in comparatively good health in her early years. In January, 1922, she had her gallbladder removed and nine months later her uterus. Menopause had occurred at forty-three.

On admission her temperature, pulse, and respiration were normal, and the patient was not acutely ill. She had many scratch marks on her back, shoulders, and arms, a shallow ulcer of two inches in diameter about the middle of the right tibia, there was moderate edema of both ankles, a scar in the right upper quadrant of the abdomen, and a midline supra pubic scar.

Examination of the heart and lungs showed no gross pathology. Her blood pressure was 145/75, red cell count, 4,490,000, white cell count, 12,200, urine showed no albumin, sugar, or casts, blood urea nitrogen, 11 mg.

No symptoms of diabetes had been elicited but her fasting blood sugar was 197 mg and on Dec. 21, 1928, the patient was transferred to the Metabolic Division.

After her admission to the Metabolic Division, the patient was subject to frequent attacks of precordial pain of anginal character, which were relieved by vasodilators. These attacks were frequent, at times more than once daily and continued for over a year. On several occasions there was an associated pulmonary congestion. Beginning about April, 1930, they became less and less frequent and she has had none since July 1, 1930.

Electrocardiographic tracings taken at various intervals always showed evidence of left ventricular preponderance and several times frequent extrasystoles.

X-ray examination of the chest showed nothing of importance except some spondylitic changes in the lower dorsal vertebrae.

There were no foci of infection found and the varicose ulcer healed in a few weeks.

On several occasions the urine showed many leucocytes but this finding did not persist and there was no other evidence of genitourinary infection. The urine at no time showed casts, the phenolsulphonephthalein excretion was normal and there was no nitrogen retention in the blood.

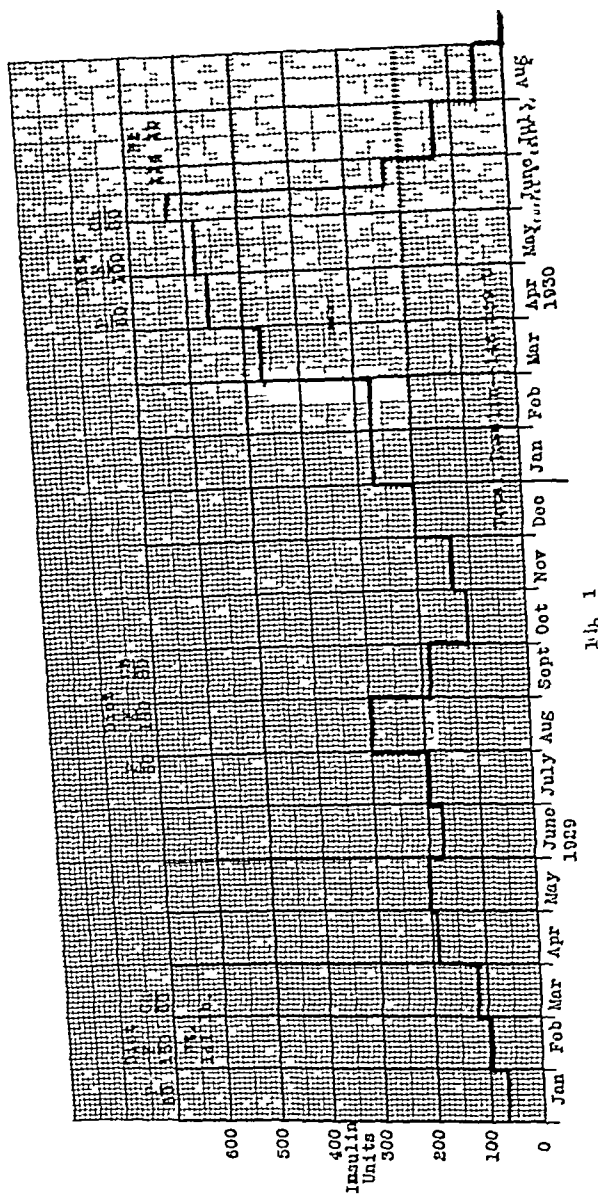
The temperature was normal throughout the period of hospitalization with the exception of a few occasions when slight rises, not above 101° F. occurred for twenty-four to forty-eight hours. These transient elevations of temperature were coincident with and apparently the results of minor ailments, such as diarrhea, bronchitis, etc.

A normal bilirubin content of the blood plasma, no retention bromsulphalein dye at the end of one-half hour and no increase of urobilinogen in the urine, indicate no liver dysfunction.

The red blood cell count varied between 5,050,000 and 3,250,000 during the period in the hospital. When discharged the count was 3,250,000. Later, on Aug. 4, 1931, the red blood cell count was 3,970,000.

The white count since admission to the Metabolic Division had varied between 5,000 and 9,700. The differential counts were normal.

Fig 1 shows a graph of the insulin requirement of the patient made by taking approximate monthly averages of insulin dosage. The decrease after the eighth month followed a change in the manner of giving the insulin



From this time on it was given at various sites over the body rather than just in the arms. This procedure may have made it more effective for a time.

During the month of March, 1930, when the refractory condition of the patient was becoming more marked, certain tests were instituted. One-tenth cubic centimeter of Lilly's U 20 pig insulin was given intradermally and a

definite wheal reaction was obtained. A supply of beef insulin was obtained and a similar sensitivity was shown. Through the kindness of Dr. E. M. K. Geiling, of Dr. Abel's laboratory, a supply of crystalline insulin was obtained. This gave the same typical skin reaction as the commercial insulin. A quantity of this crystalline insulin was dissolved sufficient for two therapeutic doses, which was given to the patient with the same local reactions as the commercial insulin. The cutaneous reaction was positive to tests repeated at intervals while the patient was in the hospital, and when tested some time after the rabbit serum had been given seemed to give even a greater reaction than formerly. For a period of about one year no cutaneous reactions were tried. In April, 1932, when tested cutaneously to Lilly's U 20 insulin, a beef insulin, and crystalline insulin, she only gave a positive reaction to the Lilly's U 20 insulin. While this was definitely positive she is no longer sensitive to crystalline insulin, and it would seem that her sensitivity is gradually disappearing. Why she was originally sensitive to crystalline insulin and now only to a commercial insulin is an interesting speculation.

In view of the large insulin requirement during March, 1930, it was thought that there must be some combination of the insulin in the patient's body. An attempt was made to ascertain whether this combination could be shown *in vitro*. The patient's serum was mixed with a quantity of insulin just sufficient to produce convulsions in a rabbit, and as a control normal serum was mixed in a like manner. These mixtures were given to various rabbits, and as far as these brief experiments could demonstrate there was no combination of the insulin with the patient's serum. Blood sugar lowering in the rabbits was just as great with the mixture of the patient's serum as with the mixture with normal serum. Three serums were procured at this time, one from a normal individual, one from a diabetic, and one from the patient. These three unlabeled serums were sent to the laboratory to be tested for precipitins. U 20 insulin was used as an antigen and the various serums were diluted. It was possible to demonstrate precipitins in the patient's blood, and it was identified in this manner from the other two serums. The results are given in Table I.

TABLE I  
SERUM DILUTIONS

SERUM	10	20	40	80	160	320	640	CONTROL
A—(Normal)	—	—	—	—	—	—	—	—
B—(Patient)	++	++	++	++	—	—	—	—
C—(Diabetic)	—	—	—	—	—	—	—	—

As a further study for allergic antibodies the Prausnitz and Kustnei passive transfer was tried. A small amount of the patient's serum was introduced intradermally into a normal person's arm. Twenty-four hours later an intracutaneous injection of a small amount of insulin was made in the same area as well as at another point, which was not sensitized. A wheal developed at the site of insulin injection in the sensitized area, while similar injection at another site was negative. This test was repeated again in April, 1932, and found to be negative.

With the thought in mind that there still was some immunologic combination of insulin with the patient's serum, a rabbit was sensitized to a mixture of 10 cc of the patient's serum and 25 units of insulin. This was done with graduated doses over a period of about three weeks. Continued attempts were made to test the patient's blood as well as this particular sensitized rabbit blood for precipitins. Insulin was used as the antigen and various dilutions of the serum were added. Although the one series of precipitin tests reported above seemed rather definite, difficulties were encountered in further trials with both the rabbit's and the patient's serum. Flocculation even occurred in the control tube and a prezone developed which later was shown to depend on the  $P_H$  of the medium. In view of these difficulties it seems desirable not to report what were first thought to be definite precipitin reactions. However, in view of the fact that there was a possibility of certain antibodies in the sensitized rabbit serum, some of this serum was given to the patient. The patient was found sensitive to the rabbit serum and was desensitized by gradually increasing doses, until finally 5 cc of the rabbit serum was injected intramuscularly. At this particular time it was necessary to give the patient over 600 U of insulin and with that dosage blood sugars were not kept under 200 mg. To make sure that the patient actually required a large amount of insulin, it was markedly reduced one day and at another time cut completely for twenty-four hours. The patient responded with a blood sugar of 320 mg,  $CO_2$  25, and a blood sugar of 328 mg  $CO_2$  23 respectively. The blood sugars were not especially high, but the acidosis was rather alarming.

About six days after the rabbit serum was given, the patient developed insulin shocks in a very surprising manner. The insulin was very rapidly cut during the next two to three days, with other insulin shocks occurring. Within a period of one to two weeks the patient's blood sugar was well controlled with from 100 to 150 U of insulin daily. After about one month at this level the insulin requirement dropped further and in October, 1930, the insulin therapy was discontinued. After insulin was totally withdrawn from the patient, blood sugars were about 90 mg. The diet therefore, was increased to contain 300 gm of carbohydrate daily. This increase was gradual and was made during a period of about five weeks. During that period the blood sugars averaged 85 mg. A few days later, November, 1930, the patient was discharged to the Out-patient dispensary and told to go on an unlimited carbohydrate intake. The dispensary blood sugars, on this regimen, averaged 115 mg. In December it was decided to do a glucose tolerance test, as the means of checking her diabetic condition. The following data were obtained

	Fasting	½ hr	1 hr	2 hr	3 hr
Blood sugar, mg	85	180	284	320	200
Urine sugar, per cent	0	—	0.6	1.6	1.0

It was obvious that the patient was still diabetic. Dispensary blood sugars for the month following the tolerance test averaged 125. The patient gained five pounds during this period.

In view of the sugar tolerance and the rather high fasting blood sugars, patient was again put on a prescription diet, containing 150 gm of carbo-

hydrate The dispensary blood sugars for the following two months averaged 126 mg with one of them 140 mg In view of these continued slightly elevated fasting blood sugars, the carbohydrate was cut to 120 gm, which diet she has remained on for the past fourteen months, until the present time This diet contains 2050 calories, and has maintained her weight rather uniformly at about 116 pounds

Fasting blood sugars over this period were usually taken at a three-week interval, and have averaged 124 mg One of these was 159 mg which immediately followed an attack of mild influenza None of these sugars during this period have been under 108 mg It would seem as if there was a tendency toward the aggravation of her diabetic condition She is an intelligent person, and we believe, tends more or less faithfully to follow her prescription diet It should be noted that during her stay in the hospital a gastric analysis showed no free hydrochloric acid present She was put upon hydrochloric acid therapy and on complaint of gastric distress during her visit to the dispensary another gastric analysis was made This showed a slight hyperchlorhydria The acid was discontinued

#### DISCUSSION

There are many interesting speculations concerning this case, with very little information as to exactly what factors were concerned in producing the interesting changes noted

The patient was very obviously sensitive to insulin itself, as far as we could determine, and not to any contaminating products of a protein nature It is generally conceded that a total diabetic would probably not need more than 100 to 200 U of insulin, under conditions where no coma or infection was present In this case, as in other cases attempts were made to find insulin in the urine, and this was unsuccessful It is quite obvious then that the insulin was either combined or destroyed in the body The tests indicate that the combination might have been of an immunologic nature It would seem to be more probable that it is a combination of this nature rather than of a lack of some substance in the tissues, such as a muscle enzyme

The sudden drop of insulin requirement might have been entirely spontaneous, but it is difficult to explain its occurring so soon after the injection of rabbit serum, if this were the case It is, of course, possible that a non-specific protein reaction produced some reaction which was responsible for this sudden drop in insulin requirement It is also possible that the rabbit serum contained some antibodies which might have combined with the substance in the patient which had previously combined with the insulin It is however, most difficult to speculate at all concerning the nature of such antibodies Therefore, it seems advisable to consider the case as one in which the refractory condition with regard to insulin was suddenly abated The added information may only induce certain considerations with regard to its cause The patient still remains sensitive to pig insulin, and is still a mild diabetic, which condition, at the present time, can be controlled by diet without insulin therapy



## SUMMARY

A case is presented of a patient who was both very sensitive and very refractory to insulin therapy. The refractory condition has disappeared, while the sensitivity persists to some extent eighteen months after withdrawal of insulin.

The patient at one time required over 600 U. of insulin daily to prevent acidosis, although the fasting blood sugars were all over 200 mg. The patient has been without insulin for eighteen months, and although still diabetic is well controlled by a prescription diet.

The refractory condition of the patient disappeared very suddenly about one week after the intramuscular injection of a rabbit serum, which may have contained antibodies, as the animal was sensitized to the patient's serum plus insulin.

We wish to express our thanks to Dr. William Kraidler, for doing the serologic work, and to Dr. Louis Tuft, for advice and aid in performing some of the immunologic reactions.

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# GLUCOSE TOLERANCE CURVES IN PULMONARY TUBERCULOSIS\*

## OBSERVATIONS UPON ONE HUNDRED CASES

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WITH THE TECHNICAL ASSISTANCE OF FREDA WARD

THE relationship between tuberculosis and diabetes is by no means clear. The existence of the two diseases in an individual is not so uncommon as one might believe. When this combination is found, the history of the diabetic condition usually precedes the onset of the tuberculosis. It is the exceptional case in which conditions are reversed, i.e., the development of diabetes mellitus in an individual who already has tuberculosis. Why the rarity? Is it possible that in tuberculosis there is present an overactivity or hyperfunctioning of the islands of Langerhans? This would no doubt give these patients a higher tolerance for carbohydrates and a lessened tendency to diabetes. Lundberg<sup>1</sup> seems to have taken this attitude. On the other hand, Langston<sup>2</sup> found that 20 of 29 patients (60 per cent) showed a Type I curve, i.e., a high blood sugar curve indicating diminished tolerance for carbohydrates. Rohdenberg, Bernhard and Kriebel<sup>3</sup> in their studies also reported that a Type I blood sugar curve was present in 60 per cent of their patients with tuberculosis. John<sup>4</sup> in his study commented on the high incidence of diabetic curves in tuberculous individuals. MacLean and Sullivan<sup>5</sup> found a normal tolerance for sugar in two children who had generalized tuberculosis.

The difference of opinion and the divergent blood sugar curves seem to indicate that the problem is a complex one. Considering the fact that diabetes is a rare development in tuberculosis we were impressed with the fact that glycosuria, on the other hand, is not uncommonly found. The blood sugar in these patients as a rule is within normal limits. In such cases, the first impression would suggest an alimentary or renal glycosuria.

We felt that further studies were warranted and hoped that a study of the blood sugar curves after the ingestion of glucose may throw some light upon the problems mentioned above.

One hundred patients suffering from pulmonary tuberculosis in various stages of the disease were selected at the Eagleville Sanatorium for the study. Two series were observed. One group (Series A) consisted of 50 patients who at one time or other showed the presence of glycosuria. Definitely established cases of diabetes were not considered. The second group (Series B) consisted of patients who never showed sugar in the urine. In other respects, the cases were unselected.

\*From the Department of Clinical Research, Eagleville Sanatorium.  
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The method of study and the interpretation of the blood sugar curves deserve some comment. After withdrawing blood, on a fasting stomach, the patient was given 1.75 mg glucose per kilogram body weight. Samples of blood were subsequently taken from the vein thirty minutes, one and two hours after the ingestion of the sugar. The technique advocated by Folin and Wu<sup>1</sup> was followed in making the blood sugar estimations.

Specimens of urine were collected before and two hours after the test for analysis.

The proper interpretation of the blood sugar curves was a problem. Unfortunately, there is no uniform classification. Suggestions for grouping the various curves have been offered by Langston, John, Hale-White and Payne,<sup>6</sup> and Mosenthal.<sup>7</sup> The types mentioned by Langston seem to be the most acceptable.

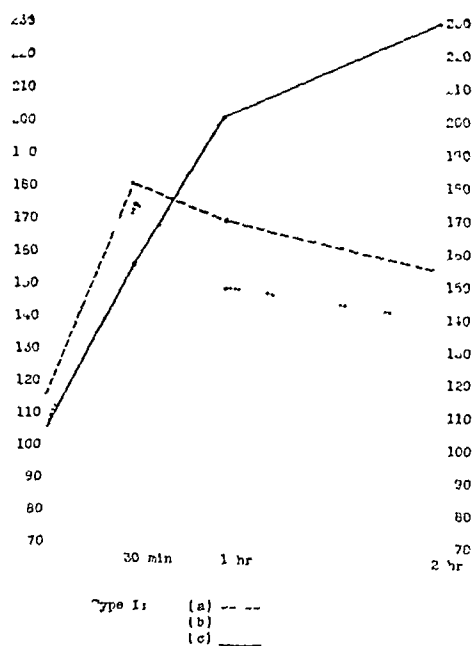


Fig 1 — Showing Type I the high or diabetic curve

He refers to the curves which reach 180 mg or above and fail to return to normal levels at the end of the two hours as Type I. The normal curve, with its fasting level between 90 and 120 mg, rises to 160 and, at the end of two hours, returns to approximately the original figure, as Type II. "Type III begins within normal limits as low or lower than in the beginning. This is the type of curve we should expect to find in hypoactivity of the thyroid, suprarenals and pituitary glands."

After collecting 100 blood sugar curves in our studies of tuberculous patients, we had some difficulty in placing them in their proper categories. Accepting Types I, II, and III suggested by Langston, subgroups were arranged so as to permit the different curves with variations to be classified more readily.

In Type I, emphasis is made not only upon the height of the rise but also the level of the blood sugar at the end of the two-hour period. This is stressed

by Brill<sup>8</sup> and Kilduffe<sup>9</sup> and seems to conform with the consensus of opinion to-day. Type I can be divided into three groups a, b, and c. In subgroup (a) the blood sugar rises to 170 or above the accepted 'spilling point' 180 mg within thirty to forty-five minutes and usually remains above this level at the end of the two-hour period, (b) the initial rise is usually between 160 and 170 mg and may drop slightly but does not return to normal limits at the end of the test, (c) the blood sugar curve has a tendency to rise steadily, continues upward and fails to drop during the period of observation. The subgroups are plotted in Fig 1.

In Type II which embraces the normal groups, the fasting blood sugar conforms to the normal limits from 90 to 120 mg. After the ingestion of glucose there is a rise at the end of thirty to forty-five minutes to about 160 mg,

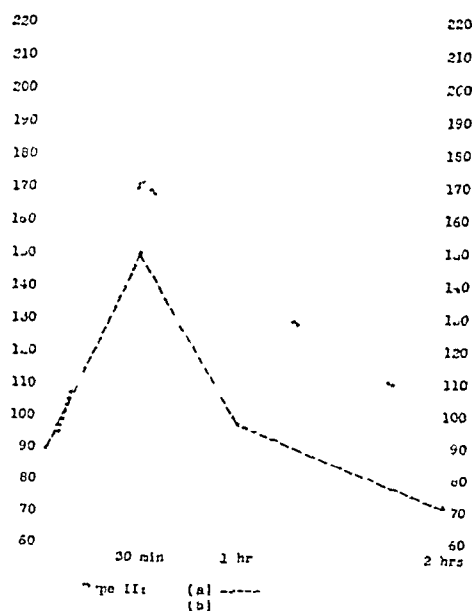


Fig 2—Showing Type II the normal curve

the blood curve then returns to the original level or slightly below at the end of the two hours. This group may be termed as Type II-a. The blood sugar in some cases rises to 170 mg or even higher, but gradually drops so that at the end of the test, the curve reaches normal limits. This group is labeled Type II-b (Fig 2).

In Type III we are dealing with curves of low range. Some are inclined to include them in the normal group. However, the mere fact that they are not diabetic curves does not necessarily mean that they are normal. When a definite amount of glucose is ingested it is presumed that a rise of blood sugar will result even if it is only temporary. Should this fail to occur then some explanation should be sought to explain this reaction which is not in line with the normal curves described above. Thaysen and Noigaard<sup>10</sup> define the low curve as one

characterized by the small height of its rise, approximately 40 mg per hundred cc or less. It is also understood that the fasting blood sugar is at the lower normal level or below. However, in the classification offered in this paper, it is suggested that we include those who may have a fasting level of 120 or 130 mg but do not rise above this, on the contrary there is a drop to lower levels after the ingestion of glucose, as evidenced by our subgroup (b). Type III therefore may be divided into three subgroups: (a) in which there is a slight rise, less than 40 mg and then a drop to lower levels, (b) in which the fasting blood sugar is within normal range and drops progressively down to subnormal levels and (c) in which the sugar curve drops below the fasting level and later rises at the end of the two-hour period but the rise is not more than 40 mg above the initial fasting level (Fig. 3).

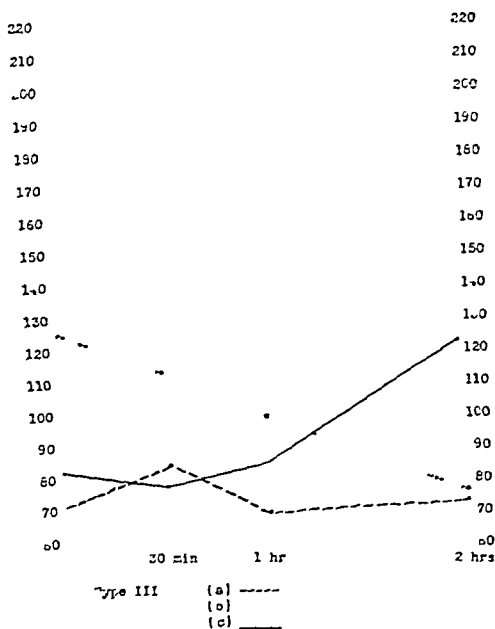


Fig. 3—Showing Type III the low curve

The blood curves were classified according to the groups or types suggested in Figs. 1, 2, and 3. Incidentally, it may be mentioned that the subgroups of the various types were selected from the sugar curves of the patients studied in the course of our observations. The following results were obtained: In Series A, there were 12 cases (24 per cent) showing Type I which may be referred to as the diabetic curve, 28 cases (56 per cent) with a normal curve or Type II, and 10 cases (20 per cent) with a low curve, Type III. Collectively, there were 38 patients (Types II and III) who had nondiabetic curves.

In Series B, the control group, there was a tendency to higher percentages of the nondiabetic curves, viz., 31 cases (62 per cent) with Type II and 14 cases (28 per cent) with Type III. Collectively, 45 patients out of 50 in this series had nondiabetic curves giving a percentage of 90 in comparison with the 76

TABLE I  
SHOWING NUMBERS AND PERCENTAGES OF THE DIFFERENT  
GROUPS IN SERIES A AND B

	SERIES A (GLYCOSURIA GROUP)		SERIES B (AGLYCOSURICS)		A AND B TOTAL
Type I Diabetic	a - 7 cases b - 2 cases c - 2 cases	12 cases (24%)	a - 1 case b - 1 case c - 3 cases	5 cases (10%)	17
Type II Normal	a - 20 cases b - 8 cases	28 cases (56%)	a - 17 cases b - 14 cases	31 cases (62%)	59
Type III Low	a - 8 cases b - - c - 2 cases	10 cases (20%)	a - 12 cases b - 1 case c - 1 case	14 cases (28%)	24

per cent in Series A. Originally our object in studying a group of controls was merely to increase the number of cases for observation. There was little to choose between the patients in both series so far as the tuberculosis was concerned. The fasting blood sugars were similar, age, sex, and basal metabolic studies varied but slightly in the two groups. The deciding factor in their classification was the presence or absence of glycosuria. In the majority of the cases, sugar was found only once. Despite this fine distinction between Series A and B, there is a higher percentage (24 per cent) of the diabetic curve and a much lower percentage of the nondiabetic curves (76 per cent in comparison with the 90 per cent) in Series A, the glycosuria group.

Another point of interest is the tendency for the patients in Series A to 'spill over' when the glucose tolerance test was performed. A glance at Table II shows 33 patients of Series A had glycosuria at the end of the two-hour period, a percentage of 66 in comparison with the 18 cases (36 per cent) of Series B.

For convenience, an analysis of the 100 cases was made in reference to age, sex, family history of diabetes, history of glycosuria, and basal metabolism. This information is arranged in Table II.

The total number of diabetic curves in both series was 17 or 17 per cent. This is much lower than the figures of Langston and Rohdenberg, et al. Langston reported 60 per cent high curves in tuberculosis with a slightly lower percentage (50 per cent) in incipient cases. Rohdenberg also mentioned finding Type I in 60 per cent of tuberculous patients.

When one considers that we may expect to find a certain percentage of high curves even in groups of apparently normal individuals and add to this the fact that in the presence of infections there is a tendency to a lessened tolerance for carbohydrates, the findings of 17 per cent high curves in tuberculosis does not seem unduly excessive.

The other side of the problem is: does tuberculosis in some way bring about an increased tolerance for carbohydrates? It is impossible to answer this question without becoming involved in lengthy discussions which are not intended in this paper. Our object in making this study in tuberculosis was to observe how these patients would react to blood glucose tolerance tests and interpret our findings accordingly.



Low blood sugar curves occur in 5 per cent of normals according to the studies of Thaysen and Nørgaard. They commented upon the dearth of literature upon this subject and reported the frequency of low blood curves in spinae and Gee-Heiter's disease. 15 of 30 patients (50 per cent) presenting this finding. In our series of tuberculous patients, the presence of the low curve in 24 per cent seems to be significant. The explanation of this is not a simple matter and various theories may be offered. Is it due to a failure of the intestinal tract to digest the sugar, is it one of the manifestations of hypofunction of the endocrine glands or is there a hyperfunction of the islands of Langerhans? In Thaysen and Nørgaard's studies on steatorrhea, they were convinced that the sugar was utilized and that the low curves could not be explained on these lines. However, their experiences were not based on tuberculous patients. Shay<sup>11</sup> noticed that in patients who had evidences of ileocecal tuberculosis, there was a failure to fully digest carbohydrates, but tuberculous patients without this intestinal complication showed complete digestion of the sugars. However, there were no cases of ileocecal tuberculosis found in this group.

It has been suggested that the low curves may be due to a hypofunction of the ductless glands. In our observations the patients showed no evidences of endocrinopathies such as myxedema or hypopituitarism. Although basal metabolic studies were not performed on all of the patients, yet we were fortunate in having some records made. Basal metabolic studies were performed on 17 of the 24 patients in Type III with the following results: 6 cases were between +1 to +10, 4 cases were between +11 to +15, 1 case was between +16 to +20, 2 cases were 0, 4 cases were between -1 to -10.

Since we cannot explain the increased frequency of this curve by a deficiency in the consumption of sugar or hypofunction of the endocrines, may it not be construed as an argument in favor of tuberculosis having some influence on the carbohydrate tolerance? Where or how this influence is exerted we are not prepared to say at this time.

The question was raised as to whether or not tuberculosis has any effect upon the sugar tolerance curve. The patients were analyzed according to the type of tuberculosis, the state of their general condition and a comparison was made with the various blood sugar curves that they presented. There were 16 incipient cases in the two series. None of the incipient cases had diabetic curves, 10 showed normal curves and 6 had low curves. Of the 64 moderately advanced cases, 13 showed high blood sugar curves of the diabetic type, 35 were in the normal group and 16 had low curves. There were 18 patients who had a fairly advanced type of tuberculosis, 4 showed diabetic curves, 12 were normal, and 2 were low.

It may be of interest to note that those patients with the milder form of tuberculosis had no high blood sugar curves. Whether this means anything or not it is difficult to say. I doubt whether too much significance may be given to this, because if so, we would expect the larger number of high curves in those patients with the most advanced forms of tuberculosis, which is not true.

As to the type of curve found in comparison with the patient's condition of the 65 patients who were improved, 7 showed diabetic curves, 39 had normal curves, and 18 had low curves. Of the 26 cases of patients whose condition was



stationary, 5 had high curves, 17 had normal curves, and 6 were low. Of the 5 retrogressing cases, 3 had high or diabetic curves and two were normal curves. Although the retrogressing cases showed higher percentages in the diabetic curves, the number is so small that I doubt whether any importance can be attached to it.

In summarizing, it appears that the incipient cases are less apt to show high curves but this may be due to the fact that this group consisted of younger individuals. The moderately advanced groups showed a slightly higher percentage of diabetic curves than the far advanced. Of the low curves, the percentage of the incipients was highest.

#### SUMMARY AND CONCLUSIONS

Blood sugar curves were studied in 100 tuberculous patients. These patients were unselected so far as the pulmonary disease was concerned.

They were divided in two groups or series. Series A included those patients who at one time or other had glycosuria. The fasting blood sugars were invariably of normal range. Diabetics were not included in this series. The control group was labeled Series B.

Suggestions of classifying the blood sugar curves are offered. They may be grouped under three main types: Type I, the high or diabetic curve, Type II, the normal curve, and Type III, the low curve.

There were 17 cases of the 100 studied showing a high or diabetic curve. Twelve of this number were found in Series A. Eighty-three cases had non-diabetic curves. Fifty-nine were normal and 24 showed low curves.

The 17 per cent diabetic curves were much below the figures mentioned by some authors.

The 24 per cent low curves impressed us as being significant.

The influence of the state of the tuberculosis upon the blood sugar curve is indefinite. Although from our experience an occasional case of increased tolerance for carbohydrates may be observed in the late stages of tuberculosis, we were unable to point out any direct bearing upon the blood sugar curves in the patients studied.

While the number of cases studied is not large to draw definite conclusions, it would seem that the low percentage of diabetic curves and the comparative high percentage of low curves may be interpreted as an indication that tuberculous patients have a higher tolerance for sugar and may explain, in a way, the rarity of diabetes developing in tuberculosis.

Grateful acknowledgment is expressed to Dr. A. J. Cohen, Medical Director of Eagleville Sanatorium, for his suggestions, courtesies, and cooperation.

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## CHOLELITHIASIS

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### A STATISTICAL STUDY WITH SPECIAL REFERENCE TO ITS FREQUENCY IN THE COLORED RACE

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R H JAFFÉ, M D, CHICAGO, ILL

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NUMEROUS statistical studies have been devoted to the frequency of cholelithiasis, and since these statistics have been collected in different parts of the world certain information can be obtained as to the racial and geographic distribution of the biliary calculi. In comparing the various statistics one meets with difficulties, however, because the material upon which they are based differs. Thus, some of the statistics have been collected from surgical clinics and laboratories while others make use of necropsies. This latter material, too, is not uniform. It is well known that below the age of twenty years, gallstones are very rare, and pathologic institutes with a large number of autopsies on children will, therefore, show a much lower percentage than institutions which draw their material from infirmaries restricted to aged and chronically disabled patients. A disadvantage of many statistics is the failure to differentiate between the principal types of gallstones, the pathogenesis of which is different. Little attention is usually given to the changes in the gall-bladder which are of much significance.

Considering first the statistics from Central Europe, in particular those from Germany, Austria, and Switzerland, the percentage varies between 8.52 (Freiburg 1 Br) and 16.6 (Vienna) (Lotzin, W Fischer, Kaufmann, Naunyn, v Recklinghausen, Schretzenmayr, a o). The average is close to 10 per cent. Scheel reported from Denmark 11 per cent while the figures for Russia are much lower, namely 2.2 per cent (E and M Hesse). In Bosnia (Balkan) Kostić counted only 1.9 per cent. The British statistics speak of from 5.4 (Middlesex Hospital—Colwell) to 8.4 (Leeds—Gross) per cent.

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\*From the Department of Pathology of the Cook County Hospital  
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For North America I quote the following figures

Mosher (Johns Hopkins)	6.91 per cent
Mitchell (Chicago Coroner)	3.1 per cent
Ophuels (St inford)	7.1 per cent
Ludlow (Cleveland)	6.97 per cent
Mentzer (Mayo)	20.09 per cent
Herter (New York Presbyterian)	7.6 per cent
Ryerson (Canada)	1.0 per cent

In Indian natives Rogers observed gallstones in 5.4 per cent. Ludlow, Mole and Van Gouda point to the rarity of gallstones among the Chinese (2.25 per cent) which also holds true for the Korean population (2.91 per cent). Statistics from Japan quote figures between 3.5 and 6.8 per cent (Miyake, Murakami, Kido).

The reports on the frequency of gallstones in the Negro race vary greatly. Garnier and Prieu assume that there are no differences in this respect between the white and the colored race. Bloch (New Orleans), Gould and Warren (Louisville), and Alden (Atlanta) are struck by the rare occurrence of biliary calculi in the Negro population of the Southern states. Mitchell did not find a case of cholelithiasis among 85 coroner autopsies on negro males. In the Canal Zone, Clark counted 2.2 per cent gallstones among negroes. In Cleveland, Ludlow found 3.08 per cent gallstones in the negroes and Mosher in Baltimore, 5.5 per cent. None of these statistics distinguishes between the different forms of stones.

In order to obtain additional information about the frequency of gallstones in the North American negro as compared to that in the white population, I have collected the data of 2621 autopsies on people over twenty years of age which were performed under my supervision between Jan. 1, 1929, and June 30, 1932. Special attention was given to the type of stone, the associated pathologic changes of the gallbladder and to the complications. The age groups below twenty were excluded since there was no case of cholelithiasis among them. The calculation of the percentage on the basis of the total number of autopsies (3633) would, therefore, have led to erroneous results.

Among the 2621 autopsies there were 208 cases with gallstones or 9.03 per cent. Arranged according to race, sex, and age the frequency of gallstones was as shown in Table I.

TABLE I

AGE	WHITE		COLORED	
	MALES	FEMALES	MALES	FEMALES
	Per Cent	Per Cent	Per Cent	Per Cent
21-30	0	5	0	3.28
31-40	1.45	11.51	0.73	7.50
41-50	4.24	21.90	0	12.24
51-60	6.75	17.07	1.86	16.27
61-70	11.20	29.03	4.34	25.0
Above 70	13.90	24.44	6.66	20.0
General Average	6.76	17.57	1.04	10.23

The most striking difference is found when one compares the figures for the white and colored males. They show that biliary calculi are six times as

common in the white males as in the negro males. For the female sex the difference is much less marked the relation between the white and colored women amounting to 1.7. Practically all the statistics on gallstones stress the higher incidence in women pointing out that the stones are from 1.6 to 5 times more common in females than in males. It is only in Japan that such sexual differences do not seem to exist (Miyake and Ishiyama). In surgical statistics the difference between males and females is even greater than in the necropsy material, the relation amounting to 1.8. Gartner and Priem suggest that in the females gallstones are more apt to cause trouble than in the males. In none of the statistics have I found such a great difference as in my group of colored patients. While the relation between the white males and females was 1.25 it was 1.10 for the colored patients.

As far as the age groups are concerned it appears that in both races gallstones occur earlier in the females than in the males in both sexes gradually increasing in frequency with progressing age.

In both races the mixed cholesterol-bile pigment-calcium stones were by far predominating, but the bile pigment stones were more common in the negroes than in the white people.

TYPE OF STONE	WHITE	COLOR
Mixed	85.18%	78.26%
Bile pigment	11.72%	17.38%
Cholesterol	3.08%	4.34%

According to Miyake and Ishiyama bile pigment stones are very common in Japan forming 65.19 per cent of the biliary calculi. In Dutch India, too, bile pigment stones are predominating (Lange).

There were no racial or sexual differences as to the number and size of the gallstones. Multiple stones were five times as common as single stones, the majority of the gallbladders containing from 10 to 25 stones. Two-thirds of the stones were from 3 to 15 mm. in diameter and only in eleven cases were the stones larger than 30 mm.

Eighty-nine (or 43 per cent) of the gallbladders with stones did not show any macroscopic changes save for an occasional decreased transparency of the wall as result of thickening of the subserous fibrous layer. Microscopically these gallbladders could not be called normal except those with pure cholesterol stones. There was a more or less marked thickening of the fibrous layer between serosa and muscularis which, in some instances, was combined with small perivascular round cell infiltrations. The muscle fibers were often slightly hypertrophic and were separated by an increased amount of connective tissue. The epithelial lined crypts of the mucosa often extended deeper into the wall than normally and the mucosa was atrophic.

The remaining 119 gallbladders revealed different forms of grossly visible changes which were classified into catarrhal cholecystitis, cholesterosis, ulcerative cholecystitis, suppurative cholecystitis, scarring of the mucosa, diffuse scarring of the wall and hydrops of the gallbladder. There were seven cases of carcinoma of the gallbladder. Among the two races and sexes the lesions of the gallbladder were distributed as shown in Table II.

TABLE II

	MACROSCOPICALLY DESCRIBED	CALCAREOUS CHOLELITHIASIS	CHOLELITHIASIS	ULCERATIVE CHOLELITHIASIS	RUPTURED CHOLELITHIASIS	SCARRING OF MUCOSA	SCARRING OF WALL	HYDROS	CARCINOMA	
White Males	49	4	3	2	8	9	3	3	3	3 cases with surgical removal of the gall bladder
White Females	27	5	3	—	7	13	12	7	2	
Colored Males	4	1	—	—	—	—	1	—	—	
Colored Females	12	3	4	—	1	4	11	3	2	

Table II indicates that the gallbladders with slight and only microscopically visible lesions are more common in the male sex than in the female sex, while in the female sex the more severe chronic lesions predominate.

In 12.9 per cent of the cases of cholelithiasis stones were also present in the extrahepatic bile ducts, namely in the common duct 17 times, in the cystic duct 13 times, in the papilla 6 times and in the hepatic duct twice. There were two cases in which the wall of the gallbladder contained numerous small faceted stones which were impacted into diverticula-like outpouchings of the wall and protruded over the serosal surface.

Perforation of the gallbladder occurred 10 times. In four instances the gallbladder communicated with the first portion of the duodenum. In two female patients with cholecystoduodenal fistulas a large stone had entered the intestine causing death from intestinal obstruction. There were two cases of pericholecystic abscesses following perforation of the gallbladder. In one instance the gallbladder had broken into the liver and had caused the formation of a large liver abscess. In another case was found an abnormal communication of the pus and stone filled gallbladder with the common duct. These two types of perforation are considered as very rare by Rolleston and McNee. One gallbladder with a ruptured aneurysm of the cystic artery had perforated into the abdominal cavity with fatal hemorrhage into the latter (S. R. Rosenthal). There was also a case of biliary peritonitis in which the bile had escaped from the tremendously distended gallbladder through several, only microscopically visible openings.

It has often been stated that gallstones are the most important predisposing factor for malignant tumors of the gallbladder and extrahepatic bile ducts. In from 60 to 95 per cent of the cases of carcinoma of the gallbladder biliary calculi have been found. Gross found gallstones in 55.5 per cent of his cases of carcinoma of the extrahepatic bile ducts. The question, however, whether the stones are not secondary to the tumor formations still remains open. In my material 70 per cent of the cases of carcinoma of the gallbladder were associated with gallstones and only 16 per cent of the carcinomas of the extrahepatic bile ducts showed this combination. The total incidence of malignant tumors of the extrahepatic bile tract (gallbladder and ducts) among the patients with gallstones was 4.29 per cent which was four times the incidence of the patients without stones. Other statistics give figures between 10.4 and 12 per cent.

In 26 instances or 12.5 per cent of the cases with biliary calculi death was directly or indirectly due to the stones. The mortality rate was decidedly higher in the white patients than in the negroes the relative figures being 18.2 and 6.2 per cent. The most common cause of death was suppurative or ulcerative inflammation of the gallbladder (16 cases). Impaction of a stone in the papilla Vateri was next in frequency (6 cases). Two cases of intestinal obstruction by gallstones and a case each of fatal hemorrhage and biliary peritonitis have been previously mentioned. Because of the still unsettled question as to the relation between cholelithiasis and carcinoma of the gallbladder and extrahepatic bile ducts I have excluded these tumors in calculating the death rate for the biliary calculi.

Because of the frequency of gallstones as a casual finding, it will be interesting to investigate whether certain extrahepatic diseases are more often associated with stone formation in the gallbladder than others. There are, especially, three conditions which have often been linked with biliary calculi, since they seem to bear evidences of a disturbed cholesterol metabolism, namely atheromatosis, carcinoma, and diabetes mellitus. Mosher found atheromatosis in 45 per cent of his cases of cholelithiasis and Chauffard observed this combination in 26.5 per cent. In Gross' material the general percentage of atheromatosis was 51.8 per cent while it was 71.8 per cent in the cases of gallstones. Pure cholesterol and mulberry stones were much more often combined with atheromatosis than the mixed stones. I am giving a list of the frequency of atheromatosis in the patients with and without gallstones, which shows that it is only in the males that slight differences can be noted (Table III).

TABLE III  
FREQUENCY OF ATHEROMATOSIS IN PATIENTS

	WITH GALLSTONES	WITHOUT GALLSTONES
White males	44.9%	32.0%
White females	20.9%	21.7%
Colored males	33.3%	19.2%
Colored females	17.5%	18.6%

Musser stated that malignancy anywhere in the body favors the formation of stones in the gallbladder and also Schietzenmayr and Lotzin point to the frequent combination of gallstones with carcinomas outside the biliary tract. Gross, on the other hand, says if anything patients with biliary calculi are slightly less liable to cancer than normal persons. Among my cases of cholelithiasis there were 40 incidences of carcinoma outside the biliary tract or 20 per cent as compared to 18 per cent in the other cases. This difference is much too insignificant as to indicate a greater disposition of the patients with gallstones to carcinoma. In this connection it may be mentioned that there exists a certain antagonism between atheromatosis and carcinoma (Casper). There were four cases of diabetes mellitus with gallstones (8.3 per cent) which is in accordance with the statement made by Rolleston and McNee that diabetes has no tendency to produce cholelithiasis (see, however, Aschoff and Gross).

Obesity has often been considered as an important predisposing factor for biliary calculi. Gross found the average body weight of the patients with gallstones to be decidedly higher than that of the patients free from stones. I refrain from giving figures of the body weight because autopsy material is of no significance in this regard.

Of the other pathologic changes those of the liver and pancreas are of importance. Biliary cirrhosis was observed in 6 cases or 3 per cent, and atrophic cirrhosis was found three times. Dufour has recently pointed to the rare combination of alcoholic cirrhosis with cholelithiasis for which he advances the odd theory that the alcohol may keep the cholesterol in solution. There were only three cases of pancreas apoplexy among the 208 patients with gallstones. The frequency of cholelithiasis in patients with cardiac diseases stressed by Brockbank is not evident in my material. I cannot comment on the statement made especially in the British literature that gallstones are particularly common among inmates of psychopathic hospitals (Candler, Flemming, Keay, *et al.*) since my observations are too limited in this regard.

#### DISCUSSION

The statistics presented in this paper indicate that in the white population of Chicago cholelithiasis is more common than in the negroes. This racial difference manifests itself chiefly in the males while in the females it is much less striking. Three factors have been associated with the etiology of the most common and most important type of gallstones, the mixed cholesterol-bile pigment-calcium stone, namely infection, biliary stasis and diets rich in fat. It has been said that the low incidence of biliary calculi in certain parts of the world is due to the vegetarian diet and strenuous outdoor life of their population. The frequency of gallstones in patients with heart diseases or depressive psychosis has been attributed to their sedentary life which favors biliary stasis.

The material upon which my observations are based is uniform and I do not think that there are sufficient differences in the diet, occupation, or mode of living between the white and colored patients of the large charity hospital of Cook County as to account for the greater disposition to biliary calculi in the white patients. It is difficult to conceive that infections of the gallbladder should be more common in the white race than in the colored race, no other infection showing such racial differences. The inability to find a satisfactory explanation in the light of the most favored theories on the formation of gallstones draws attention to additional factors which have been recently brought out by Westphal and Aschoff, namely the importance of nervous and endocrine influences upon the tonus and the emptying of the gallbladder. In the female sex the predisposing functional influences are apparently so marked that they compensate for the racial differences. Observations on the living patients, especially comparative studies on the emptying of the gallbladder between white individuals and negroes will have to decide whether the suggestions made on the basis of anatomical findings can be maintained.

## SUMMARY

A statistical study on the frequency of gallstones in the white and colored patients of a large charity hospital shows that in the white males these stones are much more common than in the negroes. In females this difference is much less pronounced. The various theories on the etiology of the gallstones are briefly discussed in the light of these findings.

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# A STUDY OF THE TOTAL AND ULTRAFILTRABLE CALCIUM AND THE ACID-SOLUBLE PHOSPHATE CONTENT OF THE BLOOD SERUM OF FOUR HUNDRED AND TWENTY-TWO HEALTHY CHILDREN\*

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A STUDY of the calcium and acid-soluble phosphate content of blood serum of a large group of children might be of some value because of the possible relationship between these values, calcium and phosphorus metabolism, and bone development. It was also thought that there might be a relationship between the incidence of dental caries and the calcium and acid-soluble phosphate content of the blood serum. The work to be reported in this paper is part of an extensive nutritional study undertaken on 422 children at Mooseheart, Illinois by the Otho S. A. Sprague Memorial Institute, the University of Chicago, and the Chicago Dental Research Club under the direction of Milton Theo Hanke.<sup>1</sup>

There were two groups of children, namely, a test group (323) and a control group (99). The test group was ingesting the standard Mooseheart diet for one year (control period) and received a pint of orange juice and the juice of one lemon per day in addition to the standard Mooseheart diet during the second year (test period). The control group received only the standard diet. The children were all living under identical conditions and they were all in good health. The total calcium and acid-soluble phosphate content of the blood serum were determined at six-month intervals during a two-year period on the test group (5 determinations per child) and during a one-year period on the control group (2 determinations per child). The ultrafiltrable calcium content of the blood serum was determined for all of the children (422) during a one-year period (2 determinations per child).

## EXPERIMENTAL METHODS

*Treatment of the Sample*—Blood (20 cc) was drawn from the median basilic vein of each of the children at six-month intervals. The blood was immediately defibrinated in a small pyrex beaker by gently stirring with a glass rod. The defibrinated blood was promptly placed in 15 cc pyrex centrifuge tubes and centrifuged for fifteen minutes, at about 1500 revolutions per minute. The clear serum was immediately removed by means of a pipette, transferred to a small pyrex tube, and transported to the laboratory.

*Determination of Total Calcium and Acid-soluble Phosphate*—Exactly 3 cc of the serum was slowly pipetted into 12 cc of 10 per cent trichloroacetic

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acid solution with constant agitation. The mixture was allowed to stand for fifteen or twenty minutes, and then filtered through a Whatman No. 40 filter paper. The clear filtrate was used for the determination of total calcium and acid-soluble phosphate.

Calcium was determined by the method of Roe and Kahn with the following modifications. Centrifugalizations were conducted for four minutes instead of two minutes as recommended by Roe and Kahn. This produces a firm mat of tricalcium phosphate which is not disturbed by the subsequent decantation and drainage. We have found it advisable to allow twenty to twenty-five minutes for drainage instead of two minutes as recommended by Roe and Kahn.

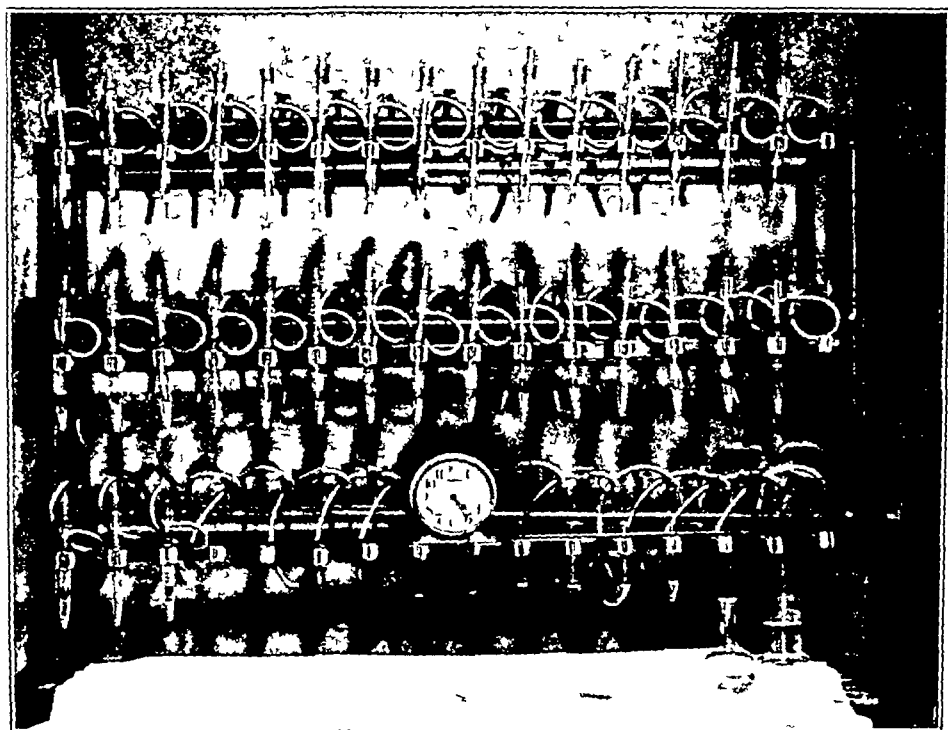


Fig. 1—View of the ultrafiltration rack showing also in the lower rack the method of formation of the membranes.

The technique and the quality of the reagents were checked each week by conducting an assay on a standard solution of  $\text{CaCl}_2$ . Powdered calcite, 62.500 mg, that has been dried for three hours at  $110^\circ \text{C}$ , is dissolved in the theoretical amount of dilute  $\text{HCl}$ . The solution is diluted to 250 cc in a Norman volumetric flask and preserved with toluene. This standard calcium solution contains 0.1 mg calcium per cc. For the test, measure 1.00 cc of this solution (by pipette) into a 15 cc graduated Pyrex centrifuge tube. Add 4 cc of a 10 per cent solution of trichloroacetic acid. Proceed from this point as in determining the calcium content of serum. The greatest average accuracy of this method is 3 per cent.

Acid-soluble phosphate was determined by the method of Fiske and Subbarow<sup>3</sup> on a 5 cc portion of the trichloroacetic acid filtrate. The color was

developed with 1, 2, 4—ammonaphthnolsulphonic acid, but this was prepared according to Roe and Kahn. There were no other modifications. The error appears to be largely colorimetric and seldom exceeds 2 per cent.

*Determination of Ultrafiltrable Ca*—The method used for the determination of the ultrafiltrable calcium content of the sera is a modification of the methods of Moritz<sup>4</sup> and of Greenberg and Gunther.<sup>5</sup>

*Apparatus*—The units of apparatus were exactly the same as those employed by Moritz. These were arranged in a specially constructed rack capable of holding 90 such units (Fig. 1). These units were then connected to the vacuum lines by means of rubber tubing equipped with pinch clamps. The vacuum lines were connected to a 30-gallon reservoir. A negative pressure of 150 mm Hg is maintained in the 30 gallon reservoir by means of a Cenco

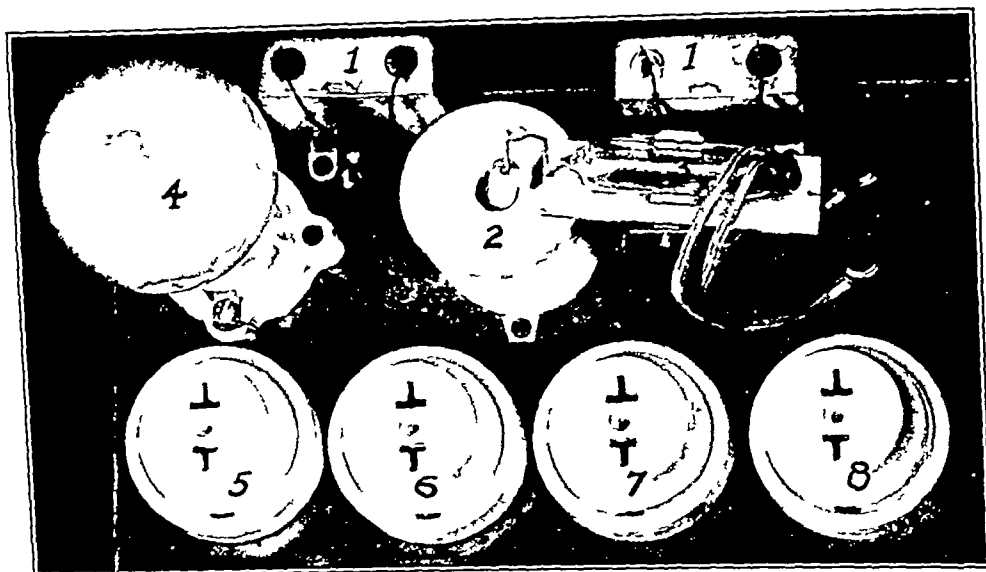


Fig. 2—Relay 1 1 mfd condenser one in each circuit 2 solenoid— $\frac{1}{4}$  lb E & S No 28 copper wire 3 Time O-Stat mercury contactor 4 40 W bulb in series with manometer contacts and solenoid 5 connection to manometer contacts 6 connection to 110 v D C manometer circuit 7, connection to 110 v A. C, pump circuit 8 connection to Cenco Megavac pump

Megavac pump. Operation of the pump is controlled by a specially designed relay that is illustrated in Fig. 2. The pressure variation was never more than  $\pm 0.5$  mm mercury.

*Membranes*—We found it difficult, at first, to prepare membranes that were uniformly permeable. The method finally adopted, which appears to be quite satisfactory and which has enabled us to prepare membranes of uniform permeability repeatedly, was as follows. Soluble cotton (nitrocellulose) which was purchased from Charles Cooper & Co., New York City, was plucked by hand and allowed to air dry overnight. It was dried at about  $40^{\circ}$  C for twelve or more hours. One hundred grams of this material was placed in a 2-liter flask, 500 cc of 95 per cent alcohol was added (the alcohol is prepared by dilution of absolute alcohol with distilled water), and then 500 cc of absolute ethyl

ether. This mixture was agitated until all of the soluble cotton had dissolved. The mixture was allowed to stand until the insoluble suspended particles had settled and then the clear supernatant liquid was decanted into 200 cc Erlenmeyer flasks, which were kept tightly stoppered until used.

The membranes are formed by pouring the soluble cotton solution in a small stream into 10 by 100 mm pyrex test tubes. Two test tubes are filled at a time. The tubes are inverted and rotated until they have almost ceased to drip, they are clamped into position as illustrated in the lower rack of Fig 2, the time is recorded and the partially solidified drippings are removed from the outlet of the test tube, from time to time, with a pair of forceps. It is imperative that the outlet be kept open throughout the drying period of exactly ten minutes. Remove the tubes from the rack and immerse in triple distilled water for thirty minutes. The transparent membranes are removed from the tubes, tested for leaks (by filling with triple distilled water, closing the orifice, and applying pressure), and cut to a length of 70 mm. The finished membranes are filled with and immersed in triple distilled water and placed in the refrigerator overnight. They are ready to use on the following day.

*The Ultrafiltration Process*—A membrane is removed from the dish and pressed in folds of clean white surgical gauze. The collapsed tube is reopened by directing a sharp forceful expiration at the orifice of the tube. Introduce exactly 3 cc of blood serum into the membrane. Lubricate stopper R\* of the ultrafiltration unit by applying a small amount of a 5 per cent solution of soluble cotton in alcohol-ether. Slip the membrane into place and tie with white silk thread. Attach the empty 15 cc graduated pyrex centrifuge tube and clamp the apparatus into place on the rack.

It was necessary, in this investigation, to conduct a large number of ultrafiltrations. The ultrafiltration units were, therefore, collected on the rack under atmospheric pressure and evacuated as a group. Ultrafiltration is continued for four hours. The filtrates should be clear and colorless and usually have a volume of 17 to 18 cc. Turbidity or color indicates leakage.

*Determination of Calcium and Acid-soluble Phosphate in the Ultrafiltrate*—Add 12 cc of a 10 per cent solution of trichloroacetic acid to the ultrafiltrate and dilute to 15 cc with triple distilled water. Mix thoroughly by repeated inversion against a clean rubber thumbcot. The calcium and phosphate content of this liquid is determined on 5 cc portions, using the methods previously described.

*Calculation of the Ultrafiltrable Calcium Value*—Rona and Takahashi<sup>6</sup> have shown that all of the phosphate of horse serum is diffusible by compensation dialysis. Neuhausen and Pineus<sup>7</sup> have shown that all of the phosphate of hog serum is ultrafiltrable. Pineus, Peterson, and Kramer<sup>8</sup> state that human serum phosphate is completely diffusible. We have conducted experiments which show that the acid-soluble phosphate of human serum is completely ultrafiltrable. We make use of this fact in the calculation of the ultrafiltrable calcium content of the serum.

\*See diagram of Moritz (loc cit.)

We have determined the total calcium and acid-soluble phosphate content of the serum and the calcium and phosphate content of the ultrafiltrate. All of the acid soluble phosphate and a certain amount of the calcium are ultrafiltrable. The ultrafiltrate will, therefore, contain a given fraction of the total acid soluble phosphate and should contain an identical fraction of the total ultrafiltrable calcium. The total ultrafiltrable calcium can, therefore, be readily calculated from the formula

$$\frac{\text{Total P}}{\text{P in ultrafiltrate}} \times \text{Ca in ultrafiltrate} = \text{Total ultrafiltrable Ca}$$

This method of calculation avoids empiric corrections for the amount of water introduced by the moist membrane, and for the volume of the proteins and other nonultrafiltrable substances that are present in the serum, because any effect that these substances may have would be equally applicable to the phosphate and to the calcium.

### RESULTS

Determinations of the total calcium and acid soluble phosphate of the blood serum of private dental patients indicated that the phosphate content of the serum became lower as a child reached maturity and the calcium content of the serum appeared to show some seasonal variation. Table I is a summary of our Mooseheart data arranged so as to show variations of the calcium and phosphate with age, and in the spring and fall of the respective years.

The calcium values, while the children were ingesting the standard Mooseheart diet, were rather consistently lower in spring than they were in fall. A

TABLE I

TOTAL CALCIUM AND ACID-SOLUBLE PHOSPHATE (CALCULATED AS P) CONTENT OF THE BLOOD SERUM OF 322 CHILDREN CHARTED BY AGE GROUPS

AGE OCTOBER, 1929	NUMBER OF CHILDREN	STANDARD MOOSEHEART DIET						STANDARD MOOSEHEART DIET PLUS 1 PINT ORANGE JUICE AND JUICE OF 1 LEMON PER DAY					
		OCTOBER, 1929 TOTAL		APRIL, 1930 TOTAL		OCTOBER, 1930 TOTAL		APRIL, 1931 TOTAL		OCTOBER, 1931 TOTAL			
		Ca	P	Ca	P	Ca	P	Ca	P	Ca	P		
		MG %	MG %	MG %	MG %	MG %	MG %	MG %	MG %	MG %	MG %		
Boys													
10	7	10.79	4.60	10.40	4.58	10.87	4.27	10.36	4.70	10.53	4.59		
11	14	10.48	4.67	10.09	4.61	10.74	4.50	10.85	4.68	10.89	4.49		
12	17	10.46	4.50	10.07	4.55	11.05	4.51	10.75	4.87	10.93	4.68		
13	23	10.41	4.65	10.14	4.76	10.77	4.44	11.05	4.76	10.62	4.50		
14	16	10.91	4.88	10.22	4.75	10.88	4.22	10.89	4.60	10.68	4.28		
15	16	10.02	4.81	9.98	4.86	10.76	4.38	10.69	4.19	10.49	3.92		
16	17	10.52	4.77	10.10	4.59	10.79	4.26	10.82	4.18	10.89	3.83		
Girls													
11	4	10.72	4.80	10.52	4.80	10.43	4.44	10.48	4.56	10.74	4.25		
12	28	11.03	4.54	10.39	4.57	10.76	4.62	10.39	4.39	10.68	4.11		
13	39	10.83	4.46	10.43	4.43	10.98	4.28	10.36	4.11	10.65	3.93		
14	46	11.13	4.23	10.32	4.17	10.99	4.16	10.46	3.99	10.68	3.92		
15	59	11.16	4.16	10.24	4.07	11.00	4.10	10.46	3.89	10.66	3.78		
16	27	10.80	3.89	10.13	3.99	10.66	3.88	10.65	3.82	10.47	3.78		
17	9	10.44	4.01	10.17	3.95	10.99	3.89	10.23	3.84	10.30	3.98		

seasonal variation in the calcium content of the serum did not occur in the case of the boys and was not very definite in the case of the girls, when orange and lemon juice was added to the daily diet. The acid-soluble phosphate content of the blood serum appears not to be subject to seasonal variations. The phosphate value is definitely lower in the more mature age groups. The values in the table suggest a fairly smooth decline in the phosphate value with increasing age in the case of the girls and no change at all in the case of the boys until they have reached the age of seventeen. Even then the decrease is not very definite in the case of the boys.

The results obtained particularly by Mellanby and Pattison<sup>9</sup> indicate that a correlation may exist between calcium and phosphorus metabolism and dental caries. We have attempted to correlate the total calcium and acid-soluble phosphate values of the blood serum with the incidence of dental caries. The results are tabulated in Table II.

TABLE II

AN ATTEMPT TO CORRELATE THE TOTAL CALCIUM AND ACID-SOLUBLE PHOSPHATE (CALCULATED AS (P) CONTENT OF THE BLOOD SERUM WITH THE INCIDENCE OF DENTAL CARIES

	NUMBER OF CHILDREN	Ca MG %	TOTAL P MG %
<i>End of Control Period (October, 1930)</i> (Standard Mooseheart diet)			
Never any caries	15	10.53	4.53
No caries for one year	52	10.94	4.28
Active progressive caries	256	10.90	4.22
<i>End of Test Period (October, 1931)</i> (Standard Mooseheart diet plus 16 oz. orange juice and the juice of 1 lemon per day)			
Never any caries	15	10.60	4.30
No caries for two years	35	10.62	4.10
Arrested caries	161	10.65	4.04
Active progressive caries	112	10.71	3.99

The values for the group marked "Never any caries" can hardly be taken as representative because there were only 15 children in this group and the individual differences were great. The values for the other groups are practically identical for any given period. We must conclude, therefore, that there is no correlation between the total calcium and acid-soluble phosphate content of the blood serum and dental caries.

It was, of course, possible that the ultrafiltrable calcium had undergone changes even though the total calcium value was identical regardless of the incidence of dental caries. We decided, therefore, to determine the amount of ultrafiltrable calcium and these determinations were conducted on all of the children during the second year of the study period. Three hundred and twenty-three of these children were receiving orange and lemon juice in addition to the standard diet during this period, 99 of the children were ingesting the standard diet but did not receive any additional citrus fruit juice. The values are summarized in Table III. It is quite obvious, from this table, that there was no

TABLE III

AN ATTEMPT TO CORRELATE THE TOTAL AND ULTRAFILTRABLE CALCIUM CONTENT OF THE BLOOD SERUM WITH THE INCIDENCE OF DENTAL CARIES

	NUMBER OF CHILDREN	APRIL, 1931		OCTOBER, 1931	
		CLIN	MG %	CLIN	MG %
		TOTAL	UIT	TOTAL	UIT
<i>Test Group</i>					
(Standard Mooseheart diet plus 16 oz orange juice and the juice of 1 lemon per day)					
Never any caries	15	10.57	5.70	10.60	6.08
No caries for two years	35	10.63	5.95	10.62	6.03
Arrested caries	161	10.60	5.81	10.67	5.99
Active progressive caries	112	10.52	5.85	10.71	6.07
<i>Control Group</i>					
(Standard Mooseheart diet No additional orange or lemon juice)					
Never any caries	3	10.50	5.53	10.41	6.76
No caries for six months	29	10.41	5.62	11.20	6.11
Active progressive caries	67	10.29	5.78	11.14	6.10

correlation between the total or ultrafiltrable calcium content of the serum and the incidence of dental caries

A comparison of the test group with the control group shows that the ingestion of orange and lemon juice had no effect upon the ultrafiltrable calcium content of the blood serum

#### SUMMARY

1 The total and ultrafiltrable calcium and the total acid-soluble phosphate content of the blood serum of a large group of children is reported

2 There were two groups of children, namely, a test group (323) and a control group (99). The test group were ingesting the standard Mooseheart diet for one year (control period) and received a pint of orange juice and the juice of one lemon per day in addition to the standard Mooseheart diet during the second year (test period). The control group received only the standard diet. The children were all living under identical conditions and they were all in good health. The total calcium and acid-soluble phosphate content of the blood serum was determined at six-month intervals during a two-year period on the test group (5 determinations per child) and during a one-year period on the control group (2 determinations per child). The ultrafiltrable calcium content of the blood serum was determined for all of the children (422) during a one year period (2 determinations per child).

3 The calcium values, while the children were ingesting the standard diet, were rather consistently lower in spring than they were in fall. A seasonal variation in the calcium content of the serum did not occur in the case of the boys and was not very definite in the case of the girls when a pint of orange juice and the juice of one lemon was added to the daily diet.

4 The acid-soluble phosphate content of the blood serum appears not to be subject to seasonal variations but is definitely lower in the more mature age groups.

5 There is no correlation between the total or ultrafiltrable calcium or total acid-soluble phosphate content of the blood serum and the incidence of dental caries.

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# A STUDY OF THE DIMETHYLAMINO BENZALDEHYDE TEST FOR ATROPINE\*†

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A NUMBER of investigations have shown that some of the color tests for the various alkaloids are not specific. Levine<sup>1</sup> has reported that many phenols give the same color reaction with selenious-sulphuric acid reagent as do some of the alkaloids. Levine and Magiera<sup>2</sup> found the same to be true for the formaldehyde sulphuric acid reaction for the opium alkaloids. Poe and co-workers<sup>3</sup> have found a number of organic compounds which give characteristic color reactions with different alkaloidal reagents.

One of the most recent tests proposed for atropine is the use of para-dimethylaminobenzaldehyde dissolved in sulphuric acid. Atropine, when treated with this reagent and heated, gives a red color which soon changes to a reddish violet. The test was proposed by Wasicky<sup>4</sup> in 1915. He tested a number of alkaloids, and found that the same test was given by hyoscyamine and scopolamine. The colors obtained by several other alkaloids were recorded. Ekkert<sup>5</sup> confirmed the work of Wasicky.

No record could be found where various organic compounds other than a few alkaloids had been tested with this reagent. The object, therefore, of the investigation reported in this paper was to test a large number of organic compounds with the dimethylaminobenzaldehyde reagent in order to determine whether or not there was any great number of substances which would give the atropine test, and whether this test was characteristic of any given organic grouping or class of organic compounds. Also, these compounds were mixed in varying amounts with atropine, and the test applied in order to determine which substances would interfere with the reaction.

## PROCEDURE

*Preparation of para-Dimethylaminobenzaldehyde Reagent*—Two grams of the aldehyde were dissolved in 6 gm of chemically pure concentrated sulphuric acid, then 0.4 cc of water was added.

*Preparation of Standard Atropine Solution*—A solution was prepared in alcohol containing one milligram of atropine per cubic centimeter.

*Preparation of Solutions to Be Tested*—The compounds to be tested were dissolved in the proper solvent so that 1 cc of the solution was equivalent to 1 milligram of the compound. Care was taken in selecting a suitable solvent which would not only readily dissolve the compound, but would also volatilize on the water-bath.

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*Procedure of Tests*—Three tests were made on each compound. For the first test, 1 c c of the solution to be tested was evaporated to dryness and then treated with two or three drops of the reagent, warmed on the water-bath for five minutes, and any color change was recorded. Any colors which developed before heating were also recorded. In the tables these are indicated with the words, "in cold." In the second test, 1 c c of the solution to be tested and 1 c c of standard atropine solution were mixed and the test made as described above. If there was no interference, a reddish violet color resulted. The third test was carried out as above, except that 5 c c of the solution of the organic compound were used with 1 c c of the atropine solution.

The results of the tests when the organic compounds were treated with the reagent are given in the pages immediately following. The name of the organic compound is given first, and directly opposite is given the color reaction. In cases where no color other than that caused by the reagents was developed, the compounds are listed with the statement, "No color reaction."

#### ALKALOIDS AND ALKALOIDAL SALTS

<i>Organic Substances</i>	<i>Color Reaction</i>
Apomorphine	Dark brown
Atropine	Reddish violet
Belladonnine	Violet in cold
Berberine	Brownish green
Brucine	Red
Cryptopine	Green
Daturine	Brownish violet
Delphinine	Brown
Emetine	Dark brown
Heroin	Brownish violet
Hyoscyamine	Reddish violet
Morphine	Rose violet to brown
Narceine	Orange
Narcotine	Brown
Papaverine	Orange
Piperine	Greenish brown
Quinidine	Brown, trace violet
Sanguinarine	Orange brown
Scopolamine	Rose violet
Solanine	Reddish violet in cold
Veratrine	Brownish violet

*No Color Reaction*—Aconitine, caffeine, cinchonidine, cinchonine, cocaine, codeine, colchicine, cotarnine hydrochloride, dionine, ergotine, letiucaine hydrochloride, gelseminine, homatropine, hydrastine, meotine, pelletierine, phenacrine, physostigmine, phloecarpine, pseudo pelletierine, quinine, sparteine, strychnine, theobromine, theophylline.

#### AMINO ACIDS AND DERIVATIVES

Acetylphenylglycine	Brown, trace violet
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*No Color Reaction*—Alpha Amino, para aminophenylglycine, arginine, asparagine, aspartic acid, *dl* benzoylalanine, betaine hydrochloride, creatine, creatinine, diiodotyrosine, edestine, ethylglycollite, glutamic acid, glycine, glycyltryptophane, hippuric acid, isoleucine, leucine, para nitrophenylglycine, alpha phenylalanine, beta phenylalanine, phenylglycine, tryptophane, tyrosine, *dl* valine

#### ALIPHATIC ACIDS

Malic acid	Brown, trace of violet
Monochloroacetic acid	Green

*No Color Reaction*—Adipic acid, alpha bromopropionic acid, beta bromopropionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malonic acid, mucic acid, palmitic acid, propionic acid, stearic acid, succinic acid, tartaric acid, trichloroacetic acid

#### ALIPHATIC ACID SALTS, ESTERS, AND DERIVATIVES

*No Color Reaction*—Ethyl oxalate, ethyl succinate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate tributyrin

#### ALIPHATIC ALCOHOLS AND KETONES

Isoamyl alcohol	Dirty violet
Isobutyl alcohol	Light green
Methylsyl alcohol	Brownish violet

*No Color Reaction*—Acetylacetone, amyl alcohol, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isopropyl alcohol, mannitol, methyl heptenone, octyl alcohol, *tert*butyl alcohol

#### SUGARS

Arabinose	Greenish black
Galactose	Black
Melezitose	Reddish brown
Xylose	Black

*No Color Reaction*—Glucose, lactose, levulose, maltose, *d* mannose, raffinose, rhamnose, sucrose

#### UREA AND URIC ACID DERIVATIVES

Allantoin	Green in cold
Barbituric acid	Brown
Triphenylguanidine	Dirty violet to black

*No Color Reaction*—Allylphenylthiocarbamide, amytal (isoamylethylbarbituric acid), acetyl methylurea, alloxantine, barbitol (diethylmalonylurea), biuret, *dl n* butylthiourea, di bromobarbituric acid, diphenylthiourea, guanine hydrochloride, ipral (calcium ethylisopropyl barbiturate), luminol (phenylethylbarbituric acid), peralga (aminopyrinediethylbarbiturate), thiobarbituric acid, thiourea, urea, urethane, uric acid

#### GLUCOSIDES

Acseulin	Dark brown
Colocythin	Brown
Elaterin	Brown
Phloridzin	Orange
Picrotoxin	Brown, trace of violet
Sabin	Reddish violet in cold
Santonin	Brown

*No Color Reaction* — Amygdalin, arbutin, convallamarin, digitalin, saponin

#### MISCELLANEOUS ALIPHATIC COMPOUNDS

*No Color Reaction* — Acetal, acetaldehyde, acetamide, acetoxime, aminoguanidine bicarbonate, bromoform, *tert* butyl bromide, chloral hydrate, chloropicrin, dimethylglyoxime, hexamethylenetetramine, iodoform, isobutylbromide, methylglyoxal sodium bisulphite, monochlorohydrine, oxamide, propionamide, sulphonal, thuridine, tribromohydrine, trimethylene bromide, trionil, veronal

#### BENZENE AND TOLUENE DERIVATIVES

*No Color Reaction* — Azobenzene, ortho bromochlorobenzene, para bromochlorobenzene, meta chloronitrobenzene, para chloronitrobenzene, para chlorotoluene, ortho dichlorobenzene, 2, 5 dichloronitrobenzene, 2, 4 dinitrochlorobenzene diphenyl, isopropylbenzene, meta nitrotoluene, ortho nitrotoluene, para nitrotoluene, styrene

#### ANILINE AND DERIVATIVES

Acetylphenetidine	Brown
Aminoazobenzene	Reddish brown in cold
meta Anisidine	Brownish black
ortho Anisidine	Brownish yellow
para Anisidine	Yellow in cold
para Benztoluide	Black
para Chloroaniline	Yellow in cold
1, 2, 4 Dinitroaniline	Rose in cold, orange with heat
Diphenylamine	Reddish brown
Diphenyl benzidine	Red
ortho Phenetidine	Reddish brown in cold
para Phenetidine	Brown
ortho Toluidine	Black
Tribromoaniline	Black
Trinitroaniline	Deep yellow

*No Color Reaction* — Acetamide, acetphenetidine, acetyl para anisidine, acetyl ortho methyltoluidine, acetyl para methyltoluidine, meta acetanilide, 2 aminotoluene 4 sulphonic acid, 2 aminotoluene 5 sulphonic acid 4 aminotoluene 2 sulphonic acid, aniline, benzamide, benzidine, ortho benztoluide, para bromoacetamide, meta bromoaniline, ortho bromoaniline, para bromoaniline, chloramine, meta chloroaniline, ortho chloroaniline, 1, 2, 4 dichloroaniline, 2, 4 dichloroaniline, ecalgine meta nitro aniline, para nitroaniline, meta nitro dimethylaniline, para nitrodimeethylaniline, nitrosodimethylaniline, 1, 2, 3 nitrotoluidine, 1, 2, 4 nitrotoluidine, 1, 3, 4 nitrotoluidine, phenyl beta diphenylamine, metatoluidine, para toluidine, 1, 2, 4 xyldine, 1, 3, 4 xyldine

#### PHENOLS AND DERIVATIVES

ortho Aminophenol	Greenish black
5 Benzalaminio 2 cresol	Black in cold
Bromothymol	Orange
ortho Chloromercuriphenol	Rose, trace of violet
ortho Cresol	Red
Oreinol	Dark brown
Resoreinol	Cherry red in cold
Thymol	Deep red
Xylenol	Brown

*No Color Reaction*—Acetyl meta aminophenol, acetyl para aminophenol, meta amino phenol, para aminophenol, benzoylthymol, para bromophenol, carvacrol, catechol, ortho chloro phenol, para chlorophenol, meta cresol, 2, 4 dichlorophenol, dimethylhydro-*o*-cresol, 2, 3 dinitro phenol, 2, 6 dinitrophenol, meta nitrophenol, ortho nitrophenol, para nitrophenol, phenol, phloroglucinol, picric acid, pyrogallie acid, tetrabromo ortho phenol, tribromophenol, trichloro phenol.

#### AROMATIC ACIDS

Naphthionic acid	Brown
Tannic acid	Black
Terephthalic acid	Violet black

*No Color Reaction*—Acetylsalicylic acid, meta aminobenzoic acid, para aminobenzoic acid, anisic acid, anthranilic acid, arsanilic acid, benzoic acid, meta bromobenzoic acid, ortho bromobenzoic acid, para bromobenzoic acid, meta chlorobenzoic acid, ortho chlorobenzoic acid, para chlorobenzoic acid, cinchophen, cinnamic acid, eumalic acid, diodosalicylic acid, 3, 5 di nitrobenzoic acid, diphenylacetic acid, gallic acid, 5 iodosalicylic acid, mandelic acid, metanilic acid, meta nitrobenzoic acid, ortho nitrobenzoic acid, para nitrobenzoic acid, salicylic acid, sulphosalicylic acid, ortho toluic acid, para toluic acid

#### AROMATIC ACID DERIVATIVES

Neocinchophen (ethyl 6 methyl 2 phenylquinoline 4 carboxylate)	Brownish green
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*No Color Reaction*—Benzamide, benzyl benzoate, butyl benzoate, coumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, methyl benzoate, methyl cinnamic ester, methyl salicylate, nicotinic acid nitrate, phenyl salicylate, phthalimide

#### AROMATIC ALDEHYDES, ETHERS, ALCOHOLS, AND KETONES

Anisole	Rose, trace of violet
Benzalacetone	Deep yellow
Benzhydrol	Brown
Benzophenone	Brown
ortho Chlorobenzaldehyde	Blood red
Guaiacol	Violet
ortho Nitrobenzaldehyde	Reddish brown
Tetramethyldiamino benzophenone	Brown
Vanillin	Dark brown

*No Color Reaction*—Para Aminoacetophenone, anisaldehyde, benzil, benzyl alcohol, 1, 2, 5 bromosalicylaldehyde, meta methoxysalicylaldehyde, phthalic acid aldehyde, salicylaldehyde, salicylaldehyde methyl ether, saligenin, para tolaldehyde

#### HETEROCYCLIC COMPOUNDS

Acridine	Light green
6 Nitroquinoline	Reddish brown
Quinoline	Dark green
Skatole	Green in cold, black with heat

*No Color Reaction*—Antipyrine, isatin, oxyquinoline, piperidine, quinidine

#### HYDROAROMATIC COMPOUNDS

Carvenone	Reddish violet in cold
Menthol	Violet black
Terpenyl acetate	Brown

*No Color Reaction*—*d* Borneol, l borneol, dl camphor (natural), camphor (synthetic), camphoric acid, camphor sulphonic acid, limonene, terpineol

#### NAPHTHALENE AND ANTHRACENE DERIVATIVES

Acet alpha naphthylide	Brownish black
Acet beta naphthylide	Greenish brown
Alizarin	Reddish brown
Dibromonanthracene	Yellowish brown
beta Naphthol	Brownish violet
alpha Naphthylamine	Greenish brown
beta Naphthylamine	Brown
alpha Naphthylaminoazo benzene	Reddish brown

*No Color Reaction*—Alpha Bromonaphthalene, 1, 5 dinitronaphthalene, beta naphthalene sulphonic acid, naphthalene anhydride, alpha naphthylisocyanate

#### MISCELLANEOUS AROMATIC COMPOUNDS

Abietic acid	Brownish violet
Dibromothymolsulphonphthalein	Reddish violet
Isoeugenole	Reddish violet
meta Nitrobenzylhydrazide	Brown
Phenanthrene	Violet black
meta Phenylenediamine hydrochloride	Brown in cold
Phenylhydrazine hydrochloride	Orange
Salarsol	Green
Thiosemicarbazide	Brownish orange
Thymolphthalein	Reddish lavender

*No Color Reaction*—Adrenaline, amirine, benzocatechin, benzoin, benzylphenylhydrazine, eugenol, 2, 4 dinitrophenylhydrazine, para nitrophenylhydrazine, phenanthrenequinone dioxide, phenolphthalein, quersite, rheumatine (saloquinine sulphate), tetrabromophenolphthalein, para tolylthiocyanate, triphenylmethane, turmeric

The color reactions for the tests where an equal amount of impurity was added to the atropine were recorded, and also the reactions where five times the amount of impurity was added, but the listing of these would require too much space. Therefore, only those reactions in which the reddish violet color was completely masked will be given. The list of substances previously given may be referred to in order to obtain the names of the compounds which did not completely cover up the atropine test. Many of these compounds gave no interference, while others gave more or less interference.

Organic compounds which completely covered up the atropine test when present in equal amounts were acetysalicylic acid, aminoazobenzene, amyl

alcohol, antipyrine, azoxybenzene, benzalacetone, benzydine, para-bromophenol, bromothymol, *dl*-*n*-butylthiourea, ortho chlorobenzaldehyde, colocynthin, dibrom-oanthracene, diphenylbenzydine, dulcitol, elaterin, eugenol, glycine, guaiacol, isobutyl alcohol, morphine, alpha-naphthylamine, beta-naphthylamine, narcotine, ortho nitrobenzaldehyde, orcinol, phloridzin, sanguinarine, skatole, sparteine, strychnine, thiobarbituric acid, para-tolyl-isothiocyanate

The organic compounds, in addition to those listed above, which completely covered up the atropine test when present in amounts five times that of atropine were acetyl-meta aminophenol, acridine, allylphenylthiocarbamide, allylthiocarbamide, para-anisidine, 5-benzalaminophenol, benzhydrol, benzophenone, carvenone, para chlorophenol, cryptopine, delphinine, 2, 4 dichlorophenol, 1, 2, 4 dinitroaniline, 1, 5-dinitronaphthalene, diphenylamine, edestine, melezitose, metanilic acid, naphthionic acid, metanitrobenzoic acid, para-toluidine, trimitroaniline, 1, 2, 4-trichloridine, xylose

From a study of the color reactions given in the tables, it will be observed that there were twenty-nine compounds which gave various shades of violet, lavender, or purple, either alone or combined with other colors. Many of these might have been mistaken for the atropine test. In general, the compounds giving a test similar to that of atropine do not belong to any definite group of organic compounds, nor does any special organic radical seem to be responsible for the characteristic test. The alkaloids as a class gave more positive reactions than any other group.

There were a few of the organic compounds which completely masked the atropine test. Others showed interference in varying degrees. Of course, in a carefully conducted analysis, many of these substances would be removed by means of the different organic solvents.

#### CONCLUSIONS

1. A number of organic compounds have been found which give a positive atropine test with para dimethylaminobenzaldehyde reagent.

2. The interferences caused by organic compounds as impurities have been determined.

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## THE SIGNIFICANCE OF MEMBRANES IN THE MUCOUS FORM OF COLITIS\*

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**I**NTRODUCTION—In a recent paper Boas<sup>1</sup> discusses colitis membranacea (or colica mucosa) as a disappearing disease and compares its incidence thirty years ago with its rarity today. This is attributed to the therapeutic measures employed in the treatment of the simple forms of colitis. Modern methods of treatment no longer include medicated and astringent enemas as important aids in combating colitis, and in Boas' opinion, the frequency of the disease has diminished in proportion to this change. Boas has found that in his recent cases of membranous colitis a history of habitual use of enemas has always been elicited. Interestingly enough the substitution of dietary treatment in place of frequent utilization of enemas was followed by the disappearance of membrane formation. It seems therefore quite clear, according to him, that membranous colitis is the result of radical treatment of simple forms of colitis with enemas and cathartics.

McCrae<sup>2</sup> speaks of mucous colitis as a remarkable disease and gives as its synonyms such names as membranous enteritis, tubular diarrhea, mucous colic, and myoneurosis intestinalis. It is a disease which has been recognized for many centuries. But a distinction must be made between the colitides associated with catarrh of the intestines and due to innumerable causes, and those due to local disease in the bowel as well as those associated with true mucous colitis. The latter is usually considered a *secretory neurosis* of the large intestine and is encountered principally in those patients (females more frequently than males) who have unstable nervous mechanisms. However, though some cases may be primarily psychogenic in origin and others organic, the majority have present both organic and psychogenic dysfunctions and even in those instances in which one factor is very much more prominent the other is doubtless contributory.

Boas<sup>1</sup> presents his views on the nature of mucous (membranous) colitis and calls attention to the many factors which may enter into its etiology. Briefly, he believes the disease may be dependent upon a primary affection of the colon in the nature of an inflammatory condition or that it may result from a secondary affection based upon a neuropathic, vagotonic state of irritation. Since the causative agent is so doubtful, it is but little wonder that the therapeutic measures employed in these cases should be at such variance. In Boas' experience, an experience which he has reason to believe is general, the disease was much more common in the latter part of the nineteenth century than it has been in the past decade. So much is this so that very little has been published

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upon this subject in the past ten years, and thus this author makes inquiry concerning the reasons for the loss of interest in this affection

In referring to the etiology of colitis membranacea, Boas believes there is definite evidence to indicate that the disease syndrome is due to a superficial catarrh involving especially the lower intestinal segment. However, the important rôle played by a disharmony in the vegetative nervous system is recognized. Nevertheless, certain facts are apparent when the superficial colitis itself is considered, and as Boas had previously pointed out, astringent substances in the form of enemas, so commonly used in the past, undoubtedly favor the formation of membranes. For example he had called attention to the fact that in patients suffering from the usual form of mucous colitis, small tannin enemas given successively in the course of a few days usually led to the formation and to the discharge of typical membranes which were previously absent, with the cessation of treatment, the formation of membranes disappeared in a short time, to reappear with the institution of astringent enemas.

Furthermore, Boas performed the following interesting experiment. Intestinal mucus was placed in a 1 to 2 per cent tannin solution and in the course of twenty-four hours a definite brown color and a membranous appearance were observed which became more pronounced in the course of the subsequent twenty-four hours. The membranes formed here did not differ histologically from the typical membrane formation in colitis membranacea or colica mucosa. Similar results were obtained when the intestinal mucus was treated with other astringent substances such as lead acetate. It is even conceivable, Boas remarks, that the addition of such harmless substances as borax, glycerin, or soap, to the enema, or even the repeated use of water enemas of an unsuitable temperature, may give rise to a catarrhal inflammation of the colon which is followed by an increased production of mucus and finally by the formation of artificial membranes.

Recently, Friedenwald and Feldman<sup>3</sup> reported upon the effect of the prolonged use of colon enemas in animals, because it was their experience that the all too frequent use of enemas and irrigations led to the production of certain colonic affections, as well as nervous intestinal disorders. In a well-controlled series of animal experiments they administered plain water, soap water, cotton seed oil, sodium carbonate solution, neutral acriflavine solution, camphor water, ammonium chloride solution, and hydrochloric acid. They concluded that enemas used over a long period of time led to more or less bowel disturbance, that even simple water enemas may produce mild inflammatory changes, that soapsuds enemas produce more marked inflammatory changes while oil enemas produce less irritation, that acriflavine (1:3000) and camphor water (U.S.P.) cause but slight disturbance of the bowel and that irritating chemicals such as sodium carbonate, ammonium chloride, and hydrochloric acid, even in weak solutions cause inflammatory changes ranging from mild processes to extensive ulcerations and necroses.

Similar observations have been made by Hirschman,<sup>4</sup> and Runge and Hartman.<sup>5</sup> Even though these findings, obtained mostly in animal experimentation, cannot be applied directly to man, it is still clear, in Boas' opinion, that the use of certain enemas, which were very popular a decade ago in the treatment of

various affections of the colon, actually favored the appearance of catarrhal changes and ulcers. Since astringent enemas are not habitually used today the reason for "colitis membranacea, a disappearing disease" becomes apparent and this disappearance is further accounted for by the fact that the irritative cathartics have been substituted by nonirritating substances.

Thus, there is established a connecting link between the so called simple colitides and the membranous type. The question therefore arises concerning the inherent value of enemas and irrigations, and in the discussion of this problem, the views of various observers are by no means in accord. In Soper's<sup>6</sup> experience "the more one irrigates, the more mucus one gets. In other words irrigations incite the secretion of mucus and if continued long enough, the 'foul smelling material' can be secured in persons with normal colons. The material is, in fact, the normal contents of the ileum when subjected to such treatment." On the other hand Bastedo<sup>7</sup> finds that "the slow, careful introduction of from six to ten gallons of water is a valuable therapeutic measure in the treatment of mucous colitis." In his conclusion, Soper remarks that "mucous colitis has been set up as an obscure and often menial disease. It is neither an inflammatory process nor a neurosis but is a spasm of the colon in which long strings of mucus are passed. The colon is an instrument that is played on by many and varied outside influences. I may mention the psychoses, gallstones, kidney stones, ulcer of the stomach and duodenum, pelvic diseases, and allergy. I have made roentgenograms showing colon spasm in the conditions mentioned. Purgative drugs, enemas, and irrigations all produce further irritation and more tendency to spasm. The underlying cause should be eradicated if possible. If the condition is not to be diagnosed, treatment should consist of retention enemas of liquid petrolatum, a smooth diet, and mild sedatives, with avoidance of all colonic manipulation."

*Experiments*—In view of Boas' work a series of experiments was planned in order to confirm or repudiate some of the opinions already briefly summarized.

First, we obtained mucus in its various forms (shreds, strings, and membranes) from some of our mucous (or mucomembranous) colitis cases, and we conducted a series of "testtube" experiments. For example, we placed bits of mucus of definite size in a bottle containing 100 cc of water. This was utilized as a control. In another bottle the same sized portions of mucus were placed but its medium was 100 cc of 2 per cent tannin solution. This experiment was repeated frequently with the following results. In twenty-four hours the water bottle showed little or no change except, perhaps, for a slight thinning of the mucus, but this may have been more apparent than real, the tannin bottle, on the other hand, seemed to contain more mucus than was originally added, but there was no increase in membrane formation. How the increased cloudiness of the tannin solution itself was to be evaluated presented some difficulty but we were well able to measure an increase in mucous surface. In forty-eight hours both solutions had become cloudy, the tannin bottle was obviously the cloudier. In these latter observations it was noted that many of the mucous particles had disintegrated, and this doubtless accounted for some of the turbidity of the solutions.

The same experiments were repeated using in place of the shreds and strings of mucus large thin membranes obtained from a patient who was under our care at the time. The membranes were placed in solutions of water, 2 per cent tannin and soapsuds. Twenty-four hours later very little change was noted in the membranes which measured two inches square. Forty eight hours later we were still unable to detect any change and for the next two days the observations were essentially negative. However, after a week it was noted that the sheets of mucus had become stiffer and thicker in the tannin medium as compared with the water medium in which they remained thin and for the most part, unchanged. A curious change takes place in the soapsuds medium where some sort of reaction between the soap and mucus results in a thick gelatinous solution but without any distinct change in the membrane of mucus itself.

It seemed, therefore that whereas tannin solution (2 per cent) did affect mucous shreds and strings so that the amount of mucous substance seemed increased, it had little or no effect on membranes, nor did it seem to form membranes even after exposure of the mucus to such a solution for a period extending over a week. The results were practically negative with water solutions and equivocal or indecisive with soapsuds solution. If 2 per cent tannin had so little effect on membranes after seven days it seemed difficult to understand the objection to its use as a therapeutic agent. It was thought, however, that the repeated use of freshly prepared 2 per cent tannin solution might cause more definite changes in membrane formation when this was carried out the results were not altogether conclusive. The mucus assumed a brown color but the membranous appearance did not become more pronounced. It was suggested, therefore, that a 10 per cent solution of tannin be utilized.

Preparations were made of thin films of mucus in 10 per cent tannin solution and as a control in water. After twenty-four hours the tannin solution was found to contain a definitely thicker and tougher piece of mucus than in the water preparation. The preparations were allowed to stand for five days and though daily the difference already described was always distinctly noted, it was found that as time passed even the mucus in the plain water assumed similar, though not as pronounced, characteristics. After ten days the mucus in 10 per cent tannin was not very hard but it was definitely coagulated while that in plain water had become soft and was easily macerated by light pressure.

Preparations of mucus in normal salt solution, 2 per cent tannin and plain water were made. Twenty-four hours later the tannin preparation was found to contain what may be described as a more durable mucus which was not increased in quantity. No change was noted in either of the remaining two media. After a week, coagulation of mucus had occurred in the tannin solution, much less marked in the salt solutions and not at all in the plain water. The same experiment was carried out with 2 per cent tannin, soap solution and plain water and the changes in the tannin and water preparations were exactly similar to those just noted. On the other hand the mucus in the soap solution seemed to have increased both in quantity and thickness (gelatinous). Daily observation over a period of five days disclosed several interesting findings. Mucus underwent gradual coagulation in the 2 per cent tannin solution, it became

tougher, thicker, and slightly shrunken. In the soap solution it assumed an apparent increased thickness due partly, it was later discovered, to the adherence of soap to the mucus. Even in plain water, mucus became a more thickly gelatinous substance.

Other solutions were used. Preparations of mucus in water, 2 per cent protargol, 1 per cent silver nitrate, 1:10,000 potassium permanganate, 1:3,000 neutral acriflavine, and  $\frac{1}{2}$  per cent sodium bicarbonate. These solutions were chosen because they are among those frequently used as enemas and irrigations in cases of colitis. After twenty-four hours it was noted, in brief, that the mucoid shreds were thinner in the water medium, somewhat thicker, and coagulated in the protargol and silver nitrate solutions, equally coagulated but slightly more resistant and indurated in the potassium permanganate solution, markedly coagulated and contracted in the neutral acriflavine and hardly changed in the sodium bicarbonate solution. After forty-eight hours the changes were essentially the same except for the neutral acriflavine solution in which the mucus had not only become markedly contracted but had been dissolved so that very little remained. Four days later the mucus (shreds and membrane) in the plain water had become thinner in the protargol, discoloration had occurred and coagulation with contraction had become more obvious, essentially the same changes took place in the silver nitrate solution as had resulted in the protargol preparation, discoloration was noted in the potassium permanganate preparation while induration and coagulation had progressed, in neutral acriflavine the changes were as already noted except that dissolution of mucus had not apparently continued, little or no change was noted in the bicarbonate preparation. After five days, observations were made on the silver nitrate and neutral acriflavine preparations. The former disclosed a considerably thickened mucus while in the latter the mucus had shrunken remarkably. After seven days the mucus in the silver nitrate solution had undergone further contraction in addition to assuming a black color. In the neutral acriflavine solution the mucus had contracted into a small hard mass but in addition the preparation contained many disintegrated bits of mucus resembling ordinary debris. The preparations were allowed to stand for six more days and during that time the mucus in the plain water preparation had become thinner but was otherwise unchanged. In the protargol solution induration, coagulation and some increase in quantity were noted. The silver nitrate preparation contained a hardened contracted mass of black material no longer resembling mucus. Hardly any further change was observed in the potassium permanganate solution except for an additional slight increase in the thickness of the membranes. The neutral acriflavine presented a picture unlike any of the other solutions. In this instance, the mucus was thickened, contracted, "gummed," granular and in parts apparently dissolved. The bicarbonate solution had not produced any change beyond a slight degree of coagulation.

These experiments, then, sum up our observations on the effect of various astringent (as well as nonastringent) solutions upon mucus, *in vitro*. Our next problem was to employ the same agents *in vivo* and to this end we produced changes resembling colitis in dogs by administering enemas of irritating solutions

such as hydrochloric acid ( $\frac{1}{2}$  to 10 per cent) and tannin (10 to 20 per cent). When the stools became diarrhea and contained an occasional trace of blood, enemas of various astringent solutions were injected.

Two dogs were given (75 c c) enemas of 10 per cent tannin solution three times a day. The stools were carefully observed. After one week had passed neither mucus nor diarrhea appeared. A 15 per cent solution of tannin was prepared and later a 20 per cent solution, but we were unable to produce either diarrhea or mucus. This experiment was abandoned. The same animals were then given 90 c c of 10 per cent hydrochloric acid per rectum once daily for two days. One of the dogs reacted by refusing his food ration and losing weight. He quickly became moribund and practically helpless and did not avoid soiled parts of his cage. The other dog, although weakened, was relatively in good health. The 10 per cent hydrochloric acid enemas were discontinued and the tannin enemas (60 to 90 c c twice daily) resumed.

After the tannin enemas had been administered for two days following the discontinuance of the 10 per cent hydrochloric acid, the sickly dog previously mentioned died. At autopsy, the entire lower bowel was found to be gangrenous, and there was a large perforation in the cecal region. The most interesting finding was concerned with the mucoid coating of the entire lower bowel. The mucus could not be peeled away in the form of membranes nor was it easy to separate it satisfactorily in any definite form except thin shreds. It adhered closely, in all its thickness, to the mucosa of the intestine. One could easily picture its possible separation as a membrane or mold of the intestine if appropriate measures were employed.

In the meantime gross and occult blood had appeared in the stools of the remaining dog and diarrhea had occurred. This was allowed to continue for four days and in the meanwhile tannin enemas were administered. They were then discontinued and for them were substituted enemas of 1 per cent hydrochloric acid (60 c c t i d). The stools became less numerous, the mucus disappeared as well as the blood. Tannin enemas and other astringent solutions were used but the results were so indefinite that it was decided to sacrifice the dog after two more weeks of such experimentation. The dog had apparently recovered completely. He was sacrificed and at autopsy the lower bowel (rectum and sigmoid colon) was found to be congested, injected, indurated, and inflamed. At the sigmoid rectal junction and for two or more centimeters below this region (almost extending to the anus in places) the mucous membrane had become so indurated that it resembled cartilage. Coincidentally two round worms (5 cm in length) were found around the appendix in one area of which the wall had become so thin as to be on the verge of perforation. Strikingly enough the entire large intestine was thickly coated with mucus, typical in appearance but difficult to separate from the mucous membrane. No ulcerated areas were found in the intestines.

In the human being, the production of excessive mucus after treatment with astringent solution has long been noted. Nevertheless, the possibility that these quantities of mucus could be the result of treatment hardly seemed to arouse serious consideration, except occasionally, until some of the more recent

reports, of which that of Boas is an example. However, long before this, many physicians had, through experience, begun to diminish the frequent use of irrigations and enemas in such conditions. Even in cases of ulcerative colitis, it was concluded by many observers that enemas often caused complications and may also have been responsible, at times, for the poor results obtained. At any rate, their use in mucomembranous colitis (comprising that entire group of mucous and membranous colitides) was doubtless more irritative than curative and seemed to favor the formation of membranes.

In *summary*, therefore, we have observed the effect of certain solutions upon mucus in vitro and in vivo, and we have been able to demonstrate that these solutions certainly bring about abnormal changes in mucous strings, shreds, and membranes in vitro. We have, however, not been able to demonstrate membrane formation with any degree of constancy in these vitro experiments. Our results confirm in many respects, the observations made by Boas. On the other hand, our vivo experiments on dogs have led us to believe that membranes are formed, but that they do not separate with ease from the intestinal wall.

*Conclusions*—As the result of our observations it is evident (1) that many solutions, and particularly those of an astringent nature, undoubtedly are irritative to the intestinal mucosa and may cause changes in the form of mucus, sometimes producing actual membrane formation. (2) Bowel irrigations and medicated enemas should therefore be utilized with the greatest caution, they should be administered only at infrequent intervals and the solutions used should always be well diluted.

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## SOME FACTORS RESPONSIBLE FOR THE SO-CALLED SELF-DISINFECTING POWER OF THE SKIN\*†

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NO SATISFACTORY explanation has been presented for the fact that bacteria die rapidly when smeared upon the surface of the skin. That pure cultures do die at a remarkably high rate was first demonstrated by Arnold and his coworkers<sup>1</sup> who showed that reductions of 90 to 100 per cent could be effected in ten to thirty minutes. They demonstrated that the condition of the skin apparently caused marked changes in the rate of reduction, as evidenced by the fact that a clean skin caused a more rapid killing action than a dirty skin. Cornbleet and Montgomery,<sup>2</sup> in a series of studies on normal and pathologic skins, demonstrated variable results, using yeast and *Staphylococcus aureus* as their organisms. They found that moist areas were less effective in destroying the test organisms than dry areas, that denuded areas were less effective than normal areas, and that, in the case of psoriasis, lesions freed of scales were more active than those with scales. These data would strongly indicate that desiccation played an important rôle, as in each instance cited above, the most successful results were obtained under conditions where desiccation was the most rapid. Systemic changes induced by the injection of gentian violet and manganese butyrate also caused changes in the rate of destruction. A depression occurred after the immediate injections, followed later by increased rates, higher than normal. No explanation is offered for the latter results. Norton and Novy<sup>3</sup> in a series of experiments using *Bacillus prodigiosus* found that bacteria dried on inert materials died at approximately the same rate as on the normal skin. They conclude that the living skin has no inherent germicidal power. Fisher<sup>4</sup> demonstrated decided fluctuations in the rate of destruction during menstrual periods when a depressed action occurred. An examination of her data shows variability from day to day and, in one instance, marked depressions at times outside the menstrual period. These marked daily fluctuations in rate of destruction suggested the work presented in this paper.

### PROCEDURE

A scientific technic for obtaining comparable results for studies of the viability of bacteria placed upon the surface of the skin or upon inert materials appears to be very difficult. No matter how the organisms are introduced upon the surface or how they are removed for examination, directly comparable results are not obtained. It seems quite reasonable to suppose that bacteria dried on a smooth surface, like glass, could be removed more readily than from an irregular surface like living skin. Furthermore, the time elapsing between smearing and

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removal of the bacteria would change the degree of attachment of the bacteria to the surface, due to the fact that the process of drying causes a closer adherence of the bacteria to the surface. Thus the longer the period of contact the greater the difficulty of removal. Granting a uniform procedure of removal, the longer the interval of exposure, the fewer organisms would be obtained. However, since similar results were always obtained under similar conditions, the data are of comparative value in measuring the effectiveness of the various procedures used. The writers deplore the lack of a more satisfactory means of measurement.

The procedure was essentially the same as that used by others. All test organisms were twenty-four-hour broth cultures. Each culture was thoroughly shaken to give an even suspension before use. An appropriate amount of the broth culture was added to sterile saline in order to obtain the proper number of bacteria. These dilutions were always prepared just prior to performing the experiments and in every case where more than thirty minutes elapsed between trials, a fresh dilution was prepared to avoid the effect of killing the organisms in the salt solution.

For convenience, the back of the hand was used for the skin tests in all cases. The hands were thoroughly washed before each experiment. Seven areas of 9 sq. cm. each were marked off with a red wax pencil. Exposures of five-tenths, one, two, three, five, ten, and fifteen minutes were used. Smears were made with a sterile cotton swab immersed in the dilution of the culture. After the proper exposure, the surviving bacteria were removed by swabbing the surface with a sterile cotton swab, previously dipped into a sterile dilution blank. The swab was then immersed in sterile saline dilution blanks of 100 c.c. amounts and shaken thoroughly. The length of time, the vigor of the swabbing, and the thoroughness of the rinsing of bacteria from the swabs were standardized in all instances to eliminate as far as possible errors due to technique. Immediately after preparing the dilutions, dextrose beef extract agar dilution plates were made. All counts were made after twenty-four hours' incubation at 37° C. *Staph aureus*, *Staph marcescens*, and *Escherichia coli* were used as test organisms.

#### EXPERIMENTAL

Norton and Novy<sup>3</sup> state that no inherent germicidal power was obtained for living skin over inert materials. Apparently, in the experiments presented these tests on skin and inert materials were made at different times with different subcultures as a source of the test organisms. To determine the significance of conducting the tests on inert material and skin simultaneously, cultures of *Esch. coli* and *Staph. aureus* were smeared on clean skin, glass, dry filter paper and moist filter paper at the same time using the same culture dilution. The tests were conducted at room temperature. Due to the fact that the bacteria frequently failed to survive an exposure of ten minutes on either inert materials or skin, intervals of five-tenths, one, two, three, five, ten, and fifteen minutes were used. Sterility, as measured by the absence of colonies on the agar plates, was obtained first on the skin, followed by dry filter paper, dry glass, and moist filter paper respectively. These data, which are representative of repeated tests, indicate that under these conditions the skin causes a more rapid destruction of the bacteria.



It may be argued that, the temperature of the skin being higher than that of the glass and filter paper, desiccation would be more rapid in the former case and that if the killing action is due to desiccation, the skin would show a greater effect. To determine this a series of experiments was conducted at 37° C so that the temperatures of all surfaces would be approximately the same. The data for *Esch. coli* are presented in Table I. It will be noted that similar results were obtained. The difference in killing action between the skin and the glass, however, is not as great, indicating that desiccation does play an important part. Similar results were obtained with *Staph. aureus* and *Seri. marcescens*, as shown

TABLE I  
THE INFLUENCE OF DESICCATION ON *ESCHERICHIA COLI* AT 37° C

TIME OF EXPOSURE	SUBSTANCE TESTED					
	SKIN		GLASS		FILTER PAPER MOIST	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
30 seconds	78,000		107,000	0	251,000	
1 min.	77,000	1.29	107,000	0	201,000	9.93
2 min.	53,000	32.06	78,000	27.11	198,000	21.12
3 min.	51,000	34.62	69,000	35.52	191,000	23.91
5 min.	800	98.98	44,000	58.88	188,000	25.10
10 min.	0	100	500	95.33	168,000	33.07
15 min.	0	100	0	100	149,000	40.64

TABLE II  
THE INFLUENCE OF DESICCATION ON *STAPHYLOCOCCUS AUREUS* AT 37° C

TIME OF EXPOSURE	SUBSTANCE TESTED					
	SKIN		GLASS		FILTER PAPER MOIST	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
30 seconds	161,000		225,000		173,000	
1 min.	133,000	17.5	230,000	-2	175,000	-1
2 min.	92,000	42.9	130,000	42.3	182,000	-4
3 min.	112,000	30.5	103,000	58.7	232,000	-32
5 min.	40,000	75.2	122,000	45.8	180,000	-2
10 min.	37,000	77.1	97,000	56.9	163,000	2.9
15 min.	34,000	78.9	97,000	56.9	170,000	1.9

TABLE III  
THE INFLUENCE OF DESICCATION ON *SERRATIA MARCESCENS* AT 37° C

TIME OF EXPOSURE IN MINUTES	SUBSTANCE TESTED					
	SKIN		GLASS		FILTER PAPER MOIST	
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
1	270,000		110,000		510,000	
1	254,000	5.93	104,000	5.46	375,000	26.87
2	16,000	94.08	122,000	-10	354,000	30.59
3	17,000	93.71	111,000	-0.9	223,000	56.28
5	6,000	97.78	95,000	13.64	165,000	67.65
10	0	100	0	100	155,000	69.61
15	0	100	0	100	141,000	72.36

in Tables II and III respectively. The data presented thus indicate that living skin does cause a more rapid destruction of bacteria than inert materials under similar conditions.

The fact that the same individual on repeated tests on successive days gives variable results may not necessarily be due to variations in technique. Marked variations do occur as demonstrated by Fisher<sup>1</sup> in her studies on the influence of the menstrual cycle. Fluctuations also occur in normal individuals from day to day. These variations are likely due to local or systemic changes of the body. Walker and Poyer<sup>2</sup> demonstrated that water irradiated with ultraviolet light had imparted to it through the exposure a residual germicidal power that was evident for several hours. An attempt was made to see if such residual germicidal powers could be produced in the skin. Accordingly, the hands of three individuals were tested for self-disinfecting power using *Serratia marcescens* and then the same hands were irradiated with ultraviolet light for one minute.

TABLE IV

INFLUENCE OF IRRADIATION BY ULTRAVIOLET LIGHT ON THE SELF DISINFECTION OF THE SKIN  
USING *SERRATIA MARCESCENS* AS A TEST ORGANISM

TIME OF EXPOSURE IN MINUTES	SUBSTANCES TESTED							
	SKIN						GLASS	
	PERSON A		PERSON B		PERSON C			
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
Before Irradiation								
$\frac{1}{2}$	224,000	0	279,000	0	218,000	0	200,000	0
1	212,000	16	286,000	0	235,000	0	191,000	5
2	192,000	15	215,000	25	191,000	28	179,000	11
5	58,000	74	81,000	71	74,000	68	168,000	17
10	38,000	82	38,000	85.4	32,000	87.2	159,000	22
15	32,000	86.2	32,000	89	36,000	85.4	134,000	38
After Irradiation								
$\frac{1}{2}$	273,000	0	261,000	0	238,000	0	254,000	0
1	232,000	14	254,000	4	241,000	0	229,000	9.8
2	229,000	16	238,000	8	219,000	13	241,000	5.1
5	10,000	96.3	13,000	50	14,000	42	184,000	27.5
10	0	100	0	100	0	94.6	152,000	37.6
15	0	100	0	100	0	100	141,000	44.5

Approximately five minutes after the exposure the same hands were tested for self-disinfecting power, using the same subculture of *Serratia marcescens*. The data are presented in Table IV. At the end of fifteen minutes' exposure of the organisms to the skin, prior to exposure to ultraviolet light, only 85 to 89 per cent of the bacteria were destroyed, whereas sterility was obtained in ten minutes after exposure to ultraviolet light. At the same time controls on glass showed only a reduction of 38 to 44.5 per cent. Repeated experiments on these same individuals and others gave similar results. Similar tests were made on chickens. The data for one such experiment are presented in Table V. The results are the same except that the difference between the counts obtained before and after irradiation was less marked. The higher body temperature of the chicken may

TABLE V

INFLUENCE OF ULTRAVIOLET LIGHT UPON SELF-DISINFECTION OF THE SKIN OF THE CHICKEN USING *SERRATIA MARCESCENS* AS A TEST ORGANISM

TIME OF EXPOSURE	GLASS		SKIN OF CHICKEN			
	COUNT	PER CENT REDUCTION	BEFORE IRRADIATION		AFTER IRRADIATION	
			COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
1 min	53,000		95,000		64,000	
1	76,000	844	73,000	21.06	15,000	76.41
3	75,000	9.65	32,000	66.32	13,000	79.69
5	75,000	9.65	15,000	84.22	10,000	84.38
10	64,000	22.90	5,000	94.74	0	100
15	65,000	21.69	0	100	0	100

have caused a more rapid evaporation of the moisture with the result that desiccation played a greater rôle and thus a less significant difference, caused by the residual action of the ultraviolet light, would be obtained

In the experiments cited, the action of the ultraviolet light was apparently a local residual effect. To determine whether or not the exposure to ultraviolet light would impart to the body an increased systemic self-disinfecting power, one hand was irradiated while the other was protected against exposure. Approximately five minutes later both hands were tested for self-disinfecting power using *Staph aureus* as a test organism. This organism was used because Cornbleet and Montgomery<sup>2</sup> failed to find that exposure to ultraviolet light had any significance as far as self-disinfecting power of the skin was concerned. The

TABLE VI

INFLUENCE OF ULTRAVIOLET LIGHT UPON SELF-DISINFECTION OF SKIN UPON PART OF BODY NOT EXPOSED TO THE DIRECT RAYS OF LIGHT USING *STAPHYLOCOCCUS AUREUS* AS A TEST ORGANISM

TIME OF EXPOSURE IN MINUTES	SURFACE TESTED							
	GLASS		HAND BEFORE IRRADIATION		HANDS AFTER IRRADIATION			
	COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION	HAND IRRADIATED		HAND NOT IRRADIATED	
					COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
1	371,000		368,000					
1	371,000	0	362,000	1.63	143,000	0	232,000	
2	369,000	0.57	349,000	5.17	146,000	0	206,000	11.22
3	367,000	1.08	299,000	18.75	144,000	0	204,000	12.07
5	367,000	1.08	294,000	22.83	75,000	47.84	204,000	12.07
10	370,000	0.27	286,000	22.29	76,000	47.14	102,000	56.04
15	368,000	0.81	203,000	44.85	6,000	96.51	53,000	55.17

data are presented in Table VI. In fifteen minutes, the skin before irradiation showed a reduction of 44.65 per cent, whereas the irradiated skin showed a reduction of 96.51 per cent. At the same time, the skin not directly irradiated showed a reduction of 55.17 per cent, an increased reduction of 10.32 per cent over the skin before irradiation. These experiments were repeatedly confirmed on the same individuals and others. Apparently as judged by these data

exposure to ultraviolet light not only causes an increased activity locally, but also imparts a systemic response

Undoubtedly the exposure to the ultraviolet light caused a reduction, as evidenced by the data, however, the mechanism of action is not known. It apparently is not due to a change of evaporation of the skin induced by the ultraviolet light exposure because the hand that was not irradiated also showed a reduction, provided the other hand had been exposed. The temperature of the light had no effect as control tests made under the same conditions without exposure to ultraviolet light caused no reduction.

Since exposure to the action of ultraviolet light imparted an increased germicidal activity to the skin, the value of sunlight was tested. These experiments were conducted in a manner similar to those cited. The hand tested was exposed to the sun by holding out of the window for fifteen minutes. In all of the experiments, the person stayed indoors prior to conducting the experiment.

TABLE VII

INFLUENCE OF SUNLIGHT UPON SELF DISINFECTION OF THE SKIN USING *SERRATIA MARCESCENS* AS A TEST ORGANISM

TIME OF EXPOSURE IN MINUTES	GLASS		SKIN			
	COUNT	PER CENT REDUCTION	BEFORE EXPOSURE		AFTER EXPOSURE	
			COUNT	PER CENT REDUCTION	COUNT	PER CENT REDUCTION
$\frac{1}{2}$	31,000	0	34,000		51,000	
1	31,000	0	32,000	5.8	29,000	43.2
2	31,000	0	32,000	5.8	29,000	43.2
3	31,000	0	33,000	2.9	21,000	58.7
5	31,000	0	32,000	5.8	6,000	88.3
10	34,000	0	800	76.5	0	100
15	30,000	3.2	0	100	0	100

to avoid previous contact with sunlight. The data are presented in Table VII. Examination of the data shows that sunlight gave results similar to those obtained with ultraviolet light.

The value of conducting the tests simultaneously is strikingly demonstrated by comparing the fluctuations in killing rates on glass in the different tables. Comparing the same organisms on different days, very marked variations were obtained. Such variables as humidity, amount of inoculum and viability of the organisms are very difficult to control, except when all the tests are made simultaneously.

#### CONCLUSIONS

- 1 Desiccation plays an important rôle in the self disinfection of the skin.
- 2 Living skin has an inherent ability to destroy bacteria implanted upon the surface.
- 3 A residual germicidal action is imparted to the skin by irradiation with ultraviolet light. Both a local and systemic reaction is obtained. The local action is more intense than the systemic action.
- 4 Exposure to sunlight causes an increased killing action similar to that obtained by irradiation by ultraviolet light.

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## THE POSSIBILITY OF A PRESSOR PRINCIPLE IN THE BLOOD OF PERSONS WITH HYPERTENSION\*

### AN EXPERIMENTAL STUDY

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THE elevation of blood pressure in arterial hypertension is due to increase of peripheral resistance in the arteriolar bed. Pathologic alteration of the arterioles sufficient in extent and degree to cause mechanically an increased resistance is not found at necropsy in patients dying of hypertension<sup>1</sup>. Spasm of the arterioles, or better, increased tonus of the arteriolar walls, which varies in degree and intensity and probably precedes structural changes, is a conception which satisfactorily explains the disturbance of the circulation in this disease.

Increased arteriolar tonus could conceivably be brought about in one or both of two ways, namely, by vasoconstrictor influences mediated through the vegetative nervous system, or by the direct action of a substance circulating in the blood stream upon the smooth muscle cells of the vessel wall or the nerve endings about them. The first hypothesis lends itself with difficulty to experimental study. With the second hypothesis in mind, investigators have repeatedly attempted to demonstrate a vasoconstrictor activity of blood or its constituents obtained from patients with hypertension. Attention has been focused upon the possibility of an hyperadrenalinemia, as suggested by Vaquez. Various biologic tests have been studied which are supposedly specific for and very sensitive to adrenalin. The positive results obtained by early workers were disproved by O'Connor<sup>2</sup> and by Hulse,<sup>3</sup> who showed that the tests are not specific and that the vasoconstrictor activity of serum may depend upon the process of clotting. However, Hulse demonstrated a "sensitizing" effect of serum obtained from patients with chronic glomerulonephritis upon the blood pressure response of the experimental animal to adrenalin. The serum of

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patients with "primary" hypertension had no such effect. Recently Kahlson and Weiz<sup>4</sup> using the rabbit ear perfusion technique, concluded that fresh blood from patients with thyrotoxicosis, fevers, and hypertension contains a vasoconstrictor principle which is not present in the blood of normal persons. In their experiments the factors of viscosity, temperature, and the possible sensitizing effects of serum were carefully controlled. These workers were not dealing with a constrictor substance the result of clotting because citrated whole blood perfused within three minutes of drawing was used. Their results are of much interest but should be corroborated.

Another method of approach to the problem is to determine the blood pressure response of the experimental animal to the injection of whole blood or serum obtained from patients with hypertension. This has received comparatively little study and the results are conflicting. Stewart and Cushing<sup>5</sup> mentioned injecting 5 to 10 cc of such serum into dogs. In three out of five experiments there resulted a fall in blood pressure, in the remaining two there was no effect. Gheorghian and Niculescu,<sup>6</sup> working also with serum, found that the blood pressure of the rabbit was uniformly raised as much as 28 mm Hg by intravenous injection of doses up to 5 cc, while the injection of similar amounts of serum from persons without hypertension did not influence the pressure. The hypertensive serum was furthermore more toxic for the rabbit than normal serum. Working with vagotomized cats "desensitized" by repeated small intravenous injections of whole blood, Danzer, Brody, and Miles<sup>7</sup> produced a pronounced rise in blood pressure which they attributed to the presence of a pressor substance in the blood of persons with hypertension. Unless the preliminary procedures of desensitization and severance of the vagi were carried out, depressor and bradycardic effects which they considered as due to anaphylactic phenomena, entirely overshadowed the otherwise obvious pressor response. Curtis, Moncrieff, and Wright,<sup>8</sup> using a similar technique, were unable to confirm these results. Injections of from 10 to 20 cc of whole blood obtained from persons with both normal and elevated blood pressures failed to produce in desensitized cats any pressor effect other than could be explained by the volume of fluid injected.

It was felt that the conflicting results of these investigators justified a repetition of these experiments. An attempt was made to allow for uncontrolled factors by making a comparatively large number of observations and comparing the results with those in a control series. Particular attention was paid to using fresh whole blood for injection, thus avoiding the formation of a vasoconstrictor substance due to clotting. This was a factor not sufficiently considered in many of the experiments described above.

#### METHOD

The carotid blood pressure of a full-grown rabbit under sodium amytal anesthesia (0.08 gm per kg by ear vein) was registered directly with a mercury manometer. The blood to be tested was drawn with a large needle from the antecubital vein and the syringe gently emptied into a flask containing enough sodium citrate to make a total concentration of 0.4 per cent. The blood was brought immediately to the laboratory and injected into the external jugu-

lar vein of the rabbit. The lapse of time from the withdrawal of the blood to its injection was usually about five minutes and never over fifteen minutes. A fresh animal was used for each determination. The amount of blood injected was 3 cc per kilogram. The lethal dose of the blood both from persons with normal blood pressure and those with hypertension was approximately the same, namely, 6 to 8 cc per kilogram. In the earlier experiments desensitization was attempted by giving repeated small intravenous injections (0.1 to 0.5 cc) before the massive dose. In some instances the animals were atropinized and the vagi were severed. These procedures did not appreciably influence the pressor response.

The patients from whom the blood was obtained were carefully studied as to renal function, degree of arterial disease, and height and lability of the blood pressure. With the exception of one patient who had malignant hypertension and renal failure, they belonged to the essential hypertension group with constantly elevated quite stable blood pressures. One patient had a coronary thrombosis resulting in a lasting fall in blood pressure. Similar curves were ob-

TABLE I

ESSENTIAL DATA IN EACH OF 20 DETERMINATIONS USING THE BLOOD OF PERSONS WITH CONSISTENTLY NORMAL OR ELEVATED BLOOD PRESSURES

NORMAL BLOOD PRESSURE	WEIGHT OF RABBIT (KILO GRAMS)	AMOUNT OF BLOOD INJECTED	DESEN- SITIZED	ATRO- PINIZED	VAGOT- OMIZED	MAXIMUM RISE IN MM. HG	DURATION OF RISE IN SECONDS
		cc					
A L	32	96	+	+	+	0	-
K F	33	99	+	+	+	0	-
A E	32	96	+	0	0	0	-
S W	27	81	+	0	0	100	25
L M	36	108	+	0	0	60	90
F B	43	129	+	0	0	0	-
E H	41	123	+	0	0	40	3
A F	37	111	0	+	0	80	45
J B	32	96	0	+	0	100	90
T A	45	135	0	0	0	100	40
						Av 48 ± 14	Av 29.3 ± 9.5
Essential hypertension							
H T	44	176	+	0	0	40	30
F M	40	120	0	±	0	80	35
E F	34	102	0	±	0	80	60
A S	38	114	0	+	0	80	60
H P	36	108	0	±	0	160	20 min
L P	34	102	0	+	0	120	40
O D	42	126	0	±	0	0	-
T L	39	117	0	±	0	120	50
A H	31	93	0	0	0	60	40
J H	40	120	0	0	0	60	15
						Av 92 ± 17	Av 153.0 ± 110
Malignant hypertension, renal failure							
J R	36	108	+	0	0	120	60

tained with his blood both before and after this occurrence. Blood from laboratory workers and medical students with normal blood pressures was used for the control determinations.

### RESULTS

In general, immediately following the injection there occurred a moderate rise in pressure of relatively short duration. This was succeeded by a pronounced fall lasting usually some minutes with eventual return to the preinjection level. The data in brief, including the height and duration of the initial rise, are given in Table I. In four of the control determinations no rise was recorded, while every rabbit in the hypertension series responded by an increase in pressure. This increase tended to persist longer in the rabbits receiving such blood than in those receiving blood from persons with normal pressures. The average rise of pressure in the control group was  $4.8 \pm 1.4$  mm. of Hg that of the

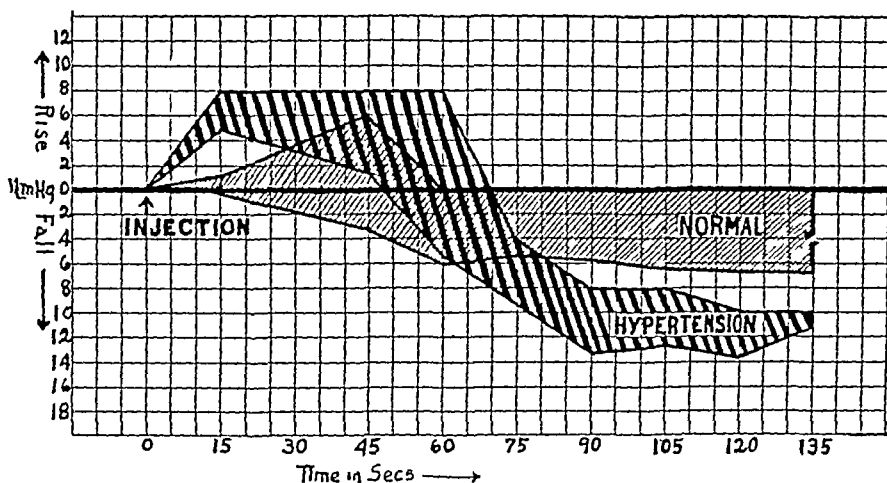


Chart 1—Blood pressure response of rabbits to the intravenous injection of whole blood from persons with normal and elevated blood pressures

hypertension group  $9.2 \pm 1.7$ , giving a difference in average values of approximately twice the standard deviation of the mean.

The scatter and overlapping of the individual observations are well shown in Chart 1, in which, for each time interval of fifteen seconds, the values lying between the average and the mode are plotted. The chart also shows the secondary fall in pressure which was more pronounced in the hypertension group.

In another limited series of experiments, the effect upon the rabbit's blood pressure of alcohol,\* ether, and water soluble constituents of blood from persons with normal and elevated pressures was determined. The detailed data will not

\*Bohn working in Volhard's clinic (see Volhard F. and Suter F. Nieren und Ableitende Harnwege, 1. p. 422 Julius Springer 1931) has uniformly produced a pronounced rise in the blood pressure of cats by the intravenous injection of alcoholic extracts of blood from patients with the 'pale' type of hypertension as typified by chronic glomerulonephritis and malignant nephrosclerosis. This finding has led Volhard to the conclusion that the elevation of pressure in such patients is the result of vascular spasm due to the presence of a 'pressor or sensitizing' substance in the circulating blood. Its exact nature remains obscure. The elevation of pressure in essential or red hypertension on the other hand depends upon a different mechanism as alcoholic extraction of the blood from such patients failed to yield a pressor principle. Our results with this procedure are in conformity with this latter finding and the patients tested belonged to the essential hypertension group.



be given because the results were uninformative. These fractions caused uniformly a transient depressor effect of approximately the same order of magnitude in all observations.

#### DISCUSSION

The nonphysiologic nature of experiments of this type, with the unavoidable introduction of uncontrollable factors of obscure nature, was recognized at the outset. It was felt that negative results would not necessarily indicate the absence of a pressor principle in the blood of persons with hypertension, whereas positive results might be significant. The results obtained, while suggestive, are statistically inconclusive because the difference in the average rise of pressure in the two series of observations does not exceed three times the standard deviation of the mean.

The secondary prolonged fall in blood pressure following injection probably represents a reaction to foreign protein which could not be obviated entirely by attempted desensitization or other procedures. That this fall was more pronounced in the group receiving the hypertensive blood is probably attributable to the fact that desensitization was attempted only once in this series as against a total of six times in the control series.

It is fair to conclude from this study that the hypothesis of a pressor principle in the blood stream of persons with consistently elevated blood pressures should not be lightly abandoned but should be subjected to further experimental study for confirmation or refutation.

#### SUMMARY

The immediate blood pressure response of the rabbit to massive intravenous injection of fresh citrated whole blood from persons with normal and consistently elevated blood pressures was studied.

In most instances a transitory rise of pressure followed by a prolonged fall resulted.

The average initial rise of pressure of the animals receiving blood from patients with hypertension was slightly greater and generally more prolonged than that observed in the control series.

Alcohol, water, and ether soluble fractions of such blood gave uniformly a transitory fall in blood pressure.

The results of this study warrant further investigation as to the possibility of a pressor principle in the circulating blood of persons with arterial hypertension.

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## MONOCYTIC LEUCEMIA\*

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SINCE the first description of monocytic leucemia by Reschad and Schilling in 1913<sup>1</sup> many similar cases have been reported in the literature, although hematologists vary in their opinions as to the acceptability of many of these cases Dameshek<sup>2</sup> in 1930 reviewed the literature on the subject and accepted sixteen of the reported cases and added two others Additional cases have been reported since that time by Lawrence<sup>3</sup> (three), Clough<sup>4</sup> (one), Cooke<sup>5</sup> (one), Farley<sup>6</sup> (one) and Asselstine<sup>7</sup> (two), bringing the total, with the three cases presented here, to twenty-nine

With the recent popularization of Pappenheim's technique for staining the leucocytes by means of neutral red and Janus green, there has been added, if not a more accurate, at least another reliable method to aid in the differentiation of leucocytic cells into the various series This has served to arouse interest in monocytic leucemia, and in several of the more recently reported cases, including two of those presented here, the supravital method of staining has been used

Since some hematologists dispute the existence of this third type of leucemia, it seems best to present as much proof as possible, in the form of carefully studied cases, that such a condition does exist

CASE 1—W I, a white male, aged sixty four years, was admitted to the University Hospital because of weakness, palpitation and moderate shortness of breath of a few weeks' duration On examination the heart and lungs were found to be normal except for moderate emphysema The lymph nodes of the inguinal, epitrochlear and axillary regions were slightly enlarged and firm in consistency Although the edge of the spleen could be felt the splenic dullness was not increased The liver was not enlarged

There was a trace of albumin in the urine The Wassermann reaction was negative Gastric analysis revealed an achlorhydria The bleeding time, coagulation time and prothrombin time of the blood were within normal limits, and the resistance of the erythrocytes to hypotonic salt solution was normal The blood platelets were diminished

The patient was given two transfusions, following which he left the hospital but returned within a week because of increasing weakness There had been no change in the physical signs except for a slight increase in the size of the superficial lymph nodes and of the liver During the next few weeks a generalized lymphadenopathy developed, the nodes being firm,

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## FOWLER MONOCYTIC LEUCEMIA

movable but not tender. The spleen and liver remained unchanged. The gums became spongy and necrotic and the face edematous. Blood cultures were sterile. The patient remained afebrile throughout. He became progressively weaker and died two months after his first admission to the hospital.

**Necropsy.** The spleen weighed 170 gm and showed a few hemorrhagic areas beneath the capsule but the splenic tissue was rather pale. The bone marrow was red with small grayish areas. The lymph nodes were enlarged and of a grayish color with small hemorrhagic areas.

On microscopic examination there was an infiltration of all organs by cells having a small amount of eosinophilic cytoplasm and a somewhat notched nucleus. In many of these only a very small bit of cytoplasm could be seen. In addition to the above cells there were neutrophils and other cells of the myelocytic series. There was a diffuse infiltration of the heart, liver, adrenals, and kidneys. The spleen showed an absence of germinal centers and the pulp was filled with the cells described above. The architecture of the lymph nodes was obliterated by the cellular infiltration and the bone marrow was likewise packed with cells of the same type.

TABLE I

DATE	HEMOGLOBIN	LYMPHOCYTES	MYELOCYTES	MONOCYTES	NEUTROPHILS	QUANTITATIVE MYELOCYTES	PLASMA CELLS	LYMPHOCYTES	UNCLASSIFIED
7/23/28	40	2,530,000	41,550	48	42			10	1
7/26/28			49,950	15	25			12	6
7/27/28	38	1,900,000	40,500	16	41	39	8	11	19
7/28/28			46,950	30	27	20	6	4	2
7/29/28				31	39	18		10	26
7/31/28	43	2,420,000	47,200	20	41	13			
8/15/28	36	1,760,000	49,800	13	51	14		22	9
8/18/28	25	1,990,000	50,900	17	58	9		7	4
8/22/28	36	1,740,000	66,700	13	53	15		15	2
8/27/28	28	2,790,000	111,000	18	17	52		11	2
9/ 1/28			124,000	32	43	23			
9/ 8/28		1,040,000	91,750						1
9/11/28	20	960,000	142,000	32	20	41		6	
9/13/28	30	790,000	151,500	21	57	18		4	
9/18/28			285,000						
9/19/28		770,000	223,000						

With Wright's stain most of the nuclei of the monocytes were bean or kidney shaped and contained a fine reticulum of chromatin without visible nucleoli. The cell borders were very irregular, sometimes frayed out and in others thrown into many folds. Small projections of cytoplasm were common, some of which were almost pedunculated. The cytoplasm contained small reddish granules. Basophilic polymorphonuclear cells were rare and no eosinophils were found. All of the myelocytes were of the neutrophilic type. An occasional normoblast was seen but these were never numerous.

At the time this patient came to the hospital we were not convinced that monocytic leucemia existed as a separate form and when first examined the diagnosis was thought to be either myelocytic leucemia or malignant lymphoma with extensive bone marrow infiltration. Repeated blood examinations, however, established the diagnosis of monocytic leucemia. The percentage of monocytes in the blood smear (48 per cent) was not as high as is reported in the majority of cases.

If the supravitral staining technique had been used in this case it is probable that many of the cells which have been classified as myelocytes on the dry smear would have been found to be of the monocytic series. The absence of basophils and eosinophils together with the size of the spleen and the necropsy report are distinctly against the diagnosis of myelocytic leucemia.

CASE 2—S A, a white male, aged forty three years, was admitted to the University Hospital on Jan 12, 1931. For five months he had noticed weakness, loss of weight, and an afternoon fever. Pustular lesions appeared on the skin but these underwent a definite change in character shortly before admission when they became firm, indurated, reddish areas without pus formation. Five days before admission he first noticed a swelling of the left lower jaw which gradually increased in size and developed an extremely offensive odor. At the same time a small tender swelling developed under the left mandible.

Examination revealed moderate pallor of the skin and mucous membranes. There was a granular, grayish mass with an indurated border and a necrotic center on the lower left gum. Extending from this lesion was an area of induration leading to an enlarged node in the submaxillary region which was tender, movable, and elastic in consistency. There were palpable nodes in the right cervical, epitrochlear, and inguinal regions. The edge of the liver could just be felt below the right costal margin but the spleen was not palpable. There were red indurated areas of varying size on the forehead, lower face, neck, back, and buttocks. The largest of these was about 3 cm. in diameter.

*Course* The lesion in the mouth progressed rapidly and involved the greater part of the lower jaw in spite of x-ray therapy. The submandibular region became swollen and very firm from below the left ear to the angle of the jaw on the right. The skin lesions increased in number, especially about the neck. Some of these became pustular while others remained as indurated areas. Similar lesions appeared in the antecubital fossa at the site of venepunctures. Soft and movable lymph nodes about 1 cm. in diameter developed in both axillae and inguinal regions. The spleen gradually enlarged until it extended two finger breadths below the left costal margin. The temperature remained at about 103° F. with only slight remissions. The patient received two blood transfusions but grew progressively worse and died Jan 27, 1931.

*Laboratory Examination* The urine showed 1 plus albumin and a few casts. The blood Wassermann was negative. Roentgenograms of the lungs and skeletal system were normal. The bleeding time, coagulation time, constrictor test, and fragility of the red blood cells were normal, but the clot retractility was poor. The platelets were slightly diminished.

TABLE II

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	MONOCYTES	BAND NEUTROPHILS	SEGMENTED NEUTROPHILS	EOSINOPHILS	MYELOCYTES	BLAST CELLS	Lymphocytes	TUBER- CULOUS CELLS	DEGENERATED CELLS	UNCLASSIFIED
1/12/31	42	3,180,000	76,200	75	3		1	3	1	10	2	5	
1/13/31			80,000										
1/18/31			51,200	75	4	3	1	4	2	6		3	2
1/19/31			50,000	72	1	3		5	3	13	1	2	
1/20/31	48	2,600,000	77,000										
1/21/31			106,000	80		5		3		6	2	4	
1/22/31			49,000	75		6		3		9	1	6	
1/23/31	40	1,730,000	107,000	84		1			3	1	1	10	
1/24/31			112,000										
1/25/31	48	2,500,000	118,000	73		5		6	2	7	2	5	

The monocytes as seen on a dry smear with Wright's stain were of two types. The first was a cell with a regular outline averaging 10 to 15 microns in diameter. It had abundant, clear, grayish lavender cytoplasm and a majority contained small, sharply defined reddish granules. A few of the cells had no granules and some had a cloudy or blotchy cytoplasm. The nucleus occupied about half of the cell area and had a definite fine chromatin network which was purplish in color. There were no large blocks of chromatin material and no visible nucleoli. The nuclei were frequently bean or kidney shaped with the cytoplasmic

granules grouped in the bend of the nucleus. Occasionally the nuclei were more irregular and in many cells it appeared to be folded on itself, producing irregular dense areas.

The second type of cell was larger in size and was more numerous. On the periphery of these cells were one or more protrusions or buds of the cytoplasm, the smaller of these being clear and without granules, while the larger ones contained reddish granules in varying numbers. A few vacuoles were found in the cytoplasm, but no inclusion of foreign material was seen. There were fewer granules in this latter type, but the cytoplasm was otherwise like that of the smaller cell. The nucleus was irregular and lobulated, and the nuclear folds were present but less evident. The nucleus stained less deeply but the chromatin network was similar to that seen in the first type. No nucleoli were seen.

Oxidase staining of the smear showed that 1.6 per cent of the white blood cells were oxidase positive, while a similar stain on a bone marrow smear showed 6.2 per cent of the cells to be oxidase positive. A suspension of bone marrow from the femur in blood serum showed that 19.1 per cent of the cells were leucocytes, whereas in a suspension from bone marrow obtained from a rib 32.1 per cent of the cells were leucocytes. This is in keeping with the fact that leucemic infiltration or metaplasia of the bone marrow is most marked in those areas which are normally most active in hematopoiesis.

Supravital staining with neutral red showed about 40 per cent of the cells to have a rosette formation of the red granules about the concavity of the nucleus. Many of the other cells had fine granules evenly distributed throughout the cytoplasm and appeared to be monocytes without the rosette. The granules and their arrangement were distinctly different from those found in the myelocytes and lymphocytes.

*Necropsy* The liver was enlarged and soft in consistency, weighing 2900 gm. Both the surface and cut section were yellowish brown in color. The spleen weighed 550 gm., was soft, and on cut section showed numerous large hemorrhages and a destruction of the normal architecture. The kidneys were enlarged and soft, weighing 360 and 420 gm. They were mottled gray in color with numerous pinpoint hemorrhages on the surface. This grayish discoloration with hemorrhagic areas was present throughout the kidney parenchyma. The myocardium was pale and flabby and there were intramuscular hemorrhages. The mediastinal and mesenteric lymph nodes were enlarged and rather firm. The bone marrow of both the femur and ribs was hyperplastic and grayish in color.

*Microscopic Examination*—The capillaries of the lungs were packed with leucocytes. These cells, for the most part, were round or oval mononuclear cells which were two or three times the diameter of the erythrocytes. The nuclei were rich in chromatin and many had irregular indented and lobulated outlines. In another section through a bronchopneumonic area an exudate was present which was not made up of polymorphonuclear cells, but consisted for the most part of the mononuclear cells described above. The heart was diffusely but unevenly infiltrated with the same type of cell.

The spleen was congested and in places there were fairly large hemorrhages. It was densely infiltrated by these large mononuclear cells, some of which had a light staining nucleus while others contained a dark staining chromatin material. Giant cells containing six or more nuclei of both the light- and dark staining types were abundant and a few mitotic figures were seen. Few neutrophils were present. In the liver the same type of cell was present with the most dense infiltration about the portal canals. The tubules of the kidneys were encroached upon by the cellular infiltration. The normal architecture of the lymph nodes was lost and the sections were made up for the most part of mononuclear cells with an occasional giant cell. Practically all organs examined showed a similar histologic picture. No evidence of proliferation of the fixed reticular tissue was found, but the infiltration by the monocytes was so dense that this proliferation, if present, might have been obscured.

CASE 3—G II, a white male, aged forty four years, was admitted to the University Hospital because of an infection in the thenar eminence of his right hand which had followed a minor injury three weeks prior to admission. He developed a fever and lymphangitis which subsided after the application of hot, moist dressings. At the time of the subsidence of the general symptoms he noticed excessive salivation, and a swelling of the left cheek. Four days

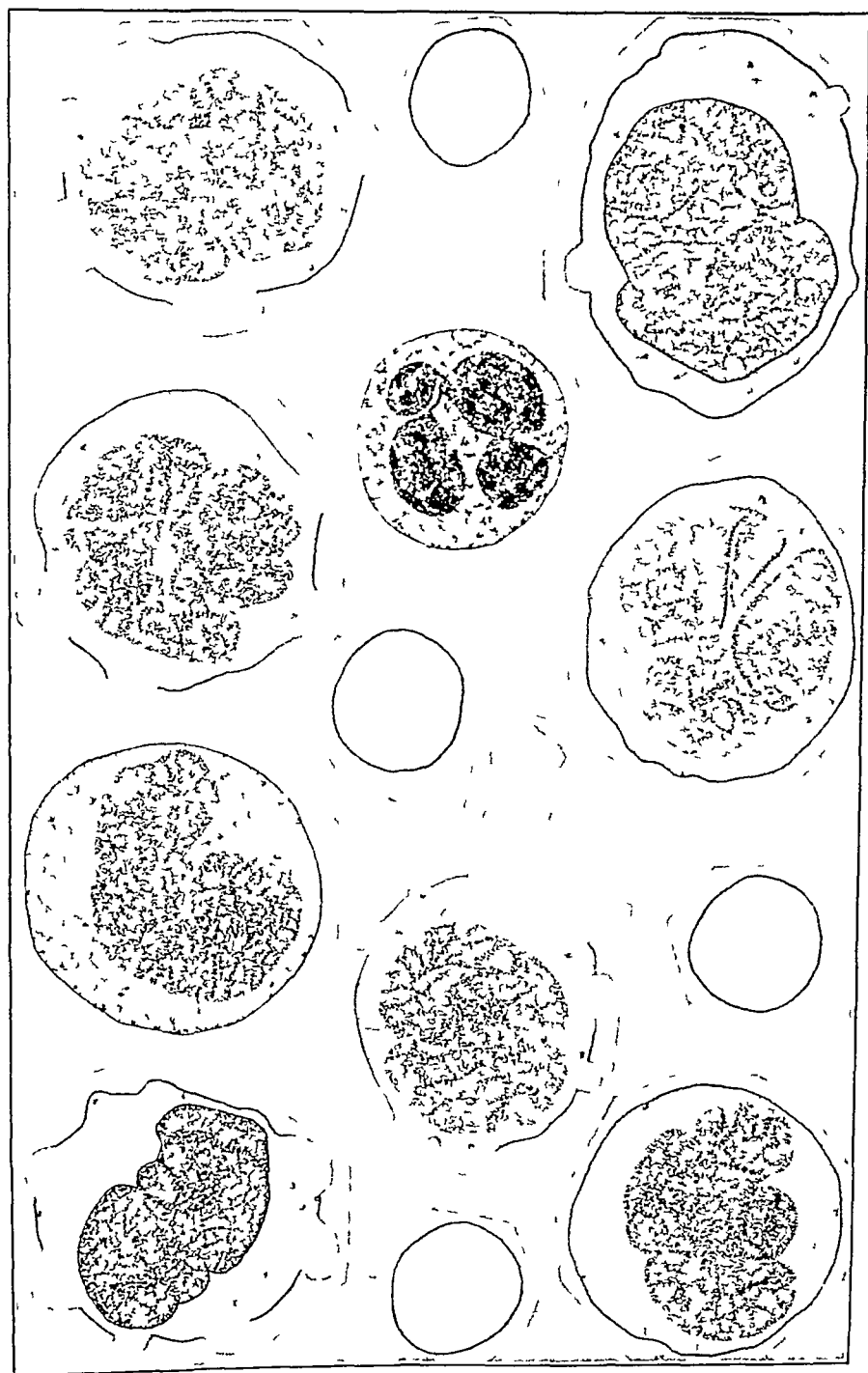


Fig 1—Camera lucida drawings of monocytes with erythrocytes and one neutrophil for comparison. Made from a fixed smear with Wright's stain.

later a similar painful swelling appeared on the right side of the face which gradually increased while that on the left disappeared. An abscess on the thenar eminence of the right hand was incised and a small amount of pus liberated.

The patient was extremely weak and at times he was semicomatose or irrational. There was marked pallor of the sclera, skin and mucous membranes but there were no petechiae or other evidences of hemorrhage. There was a swelling of the right cheek extending downward from the malar eminence over the face and submandibular region. The most prominent part of the swelling was just below the level of the line of closure of the teeth. This area was definitely tender to touch and was firm and indurated, but there was no discoloration of the overlying skin. The submandibular nodes were enlarged and firm on both sides but the enlargement was greatest on the right. There was excessive salivation and a very foul odor to the breath. On the inner surface of the right cheek was a grayish brown area of necrosis 1.5 by 3 cm in diameter. The margins of this area were clear cut and there was no induration, discoloration or other evidence of infection in the surrounding tissue and it appeared to be purely a necrotic lesion. The inner surface of the cheek was diffusely swollen. The gingivae were swollen, infiltrated, and bled rather easily with pressure, although there were no spontaneous hemorrhages. The gingivae extended upward between the teeth as high as the incisor surfaces in some places.

The posterior cervical, epitrochlear, axillary, and inguinal nodes were enlarged but remained discrete and movable. The cardiorespiratory system was normal. The liver and spleen were not palpable although the area of splenic dullness was definitely increased. At the base of the thenar eminence of the right hand was a red, indurated area with an incision in the center from which a small amount of seropurulent material could be expressed.

*Course*—During his first few days in the hospital the patient had a fever which reached 102° F on several occasions, but later the temperature became subnormal. A blood culture remained sterile. The patient's skin was very warm and moist at all times regardless of his temperature, and he had frequent, drenching sweats. The lesion on the hand improved but those on the gums and cheek remained stationary. He became progressively weaker and the

TABLE III

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	MONOCYTES	NEUTROPHILS	LYMPHOCYTES	PLASMA CELLS	MYELOCYTES	BLEAST CELLS	PLASMA CELLS	TUBERCLE IRITATION CELLS	DEGENERATED CELLS	UNCLASSIFIED	REMARKS
10/17/31	65	2,800,000	16,000											
10/18/31			29,650	23	55							5		
10/20/31		2,500,000	40,000	64	22							3		
10/21/31			32,000	74	3				1			5		
10/22/31	35	1,690,000	13,850	71	6						22	1		
11/23/31	35	2,230,000	22,450	88	15						9	1		
10/24/31	35	2,250,000	25,280	72	3						15	10		
10/25/31			28,350	75	2				2		12	9		
10/26/31			35,200											Trans fusion 520 cc
10/27/31	35	1,770,000	10,250	56	17		2		3	13		8	1	
10/28/31	40	2,090,000	19,600	65	9			1	4	11		10		
10/29/31			12,200											
10/30/31			11,200											Trans fusion 300 cc
10/31/31			13,400	54	11	1	1			23		10		
11/ 1/31			13,200	71	7					16		6		
11/ 2/31			11,000	55	11				5	15		14		
11/ 3/31				64	10				1	17		8		

erythrocyte count decreased despite two transfusions. He was mentally depressed and finally demanded his discharge from the hospital. He died at his home on Nov. 23, 1931, about nine weeks after the onset of symptoms.

**Laboratory Examination**—Urinalysis showed a trace of albumin but no casts. The blood uric acid was 5.7 mg per cent, urea nitrogen 14.7, creatinine 1, and cholesterol 595. A roentgenogram of the mandible showed no evidence of osteomyelitis. The hematocrit reading was 27 per cent and the blood platelets were normal.

On a fixed smear the predominant cell in this case varied from 10 to 15 microns in diameter, with the nucleus occupying three fourths or more of the cell area. The configurations of the nuclei were extremely variable, a few were almost circular in outline, but the great majority were very irregular. Even in those with the most regular outline there was usually some degree of indentation, and practically none had a uniform density throughout the nucleus. The nucleus gave the appearance of being folded on itself so that there were sharply outlined dense areas, the outline of which could frequently be followed out to the periphery of the nucleus and found to coincide with a curve of the nuclear outline. Some of the cells had



Fig. 2—Camera lucida drawings of monocytes with erythrocytes for comparison. One monocyte is seen with the inclusion of an erythrocyte. Made from a fixed smear with Wright's stain.

a horseshoe shaped nucleus which frequently contained nuclear folds. The nuclei in all these cells took a bluish purple tint with Wright's stain. The internal structure consisted of a definite reticular network with rather dense chromatin threads. There was no tendency of the chromatin to form in blocks and nucleoli were extremely rare.

The cytoplasm was basophilic and varied in density from a light hyaline to a dark blue color which was blotchy or coagulated in appearance. There was no perinuclear clear area. In some cells there was only a very narrow rim of cytoplasm, usually visible in two or more places opposite nuclear indentations. A few fusiform cells were present and on a few cells there were small rounded cytoplasmic projections with indistinct outlines which suggested pseudopodia. For the most part the cytoplasm was agranular although a few had small bluish granules. One of these cells was found on a fixed smear with an engulfed red blood cell.

With the vital stains the monocytes were found to have a very indistinct cell outline with many small cytoplasmic protrusions, pseudopodia and elongated processes which were frequently drawn out to a point. The pseudopodia, when present, were at the opposite side of the cell from the nucleus.

The nucleus in most cells was large and irregular in outline and occupied about three fourths of the cell body.



With neutral red the granules were distinctly different from those of the granulocytic series. The number of granules in the monocytes varied, but for the most part they were numerous and were commonly grouped at one side or in the bend of the nucleus. A few did not have this characteristic rosette, but showed granules scattered throughout the cytoplasm and some cells had a few granules scattered in other areas outside the rosette. The polymorphonuclear granulocytes were extremely active, while motion in the monocytes was sluggish. Large red vacuoles appeared in the cells after a long period of observation.

With Janus green only a few scattered mitochondria were seen. The number, size, and distribution of these did not correspond to those of the lymphocytes, and we were unable to trace any gradation indicating that there were transitional stages between the monocytes and lymphocytes. No cells were found with the very numerous, small mitochondria characteristic of the myelocyte nor were transitional stages between the monocyte and granulocyte noted.

Vacuoles were present in many cells, but none were found with incorporation of other cells or cell fragments, although one such cell was seen on a fixed smear. No amitotic or mitotic figures were seen.

With a combination of Wright's and peroxidase stain on a fixed smear, it was found that 63 per cent of the monocytes contained the peroxidase ferment while 37 per cent had none.

TABLE IV  
DIFFERENTIAL COUNT BY MEANS OF NEUTRAL RED

	10/22/31	10/24/31	11/2/31	11/3/31
Monocytes	73	84	55	56
Lymphocytes	22	10	11	12
Granulocytes	5	6	34	32

TABLE V  
PEROXIDASE CONTENT OF CELLS

	10/21/31	10/25/31	10/28/31	11/1/31	11/3/31
Much	2	2	11	2	2
Little	16	30	40	40	38
None	82	68	49	58	60

The cells of the last two cases presented several points of dissimilarity and by the existing criteria those in the third case would be classified as the more immature forms of the monocytic series. These cells (Case 3) were somewhat smaller, the cytoplasm was more basophilic and less granular, pseudopodia were less in evidence, and nucleoli, while not common, were more numerous than in the cells of Case 2. We were unable to discern with either Wright's, peroxidase, or supravital stains any convincing evidence of transition between these cells and either lymphocytes or myelocytes.

No conclusions as to the incidence of this condition can be drawn from the number of cases reported in the literature except that it is a relatively rare form of leucemia. There have been admitted to the University Hospital during a fifteen-year period 782 cases of diseases involving primarily the hematopoietic system. Of this group, monocytic leucemia forms only 0.38 per cent, and of the 164 cases of leucemia, it represents 1.82 per cent. There were 28 cases of lymphocytic and 20 cases of myelocytic leucemia to each case of the monocytic form.

The etiology of monocytic leucemia is unknown, and although certain features suggest an infectious basis, as in the case of other forms of leucemia, the neoplastic origin seems most probable.

Certain infectious processes closely simulate the various forms of leucemia and must always be considered in the differential diagnosis. This differentiation is likely to be most difficult in acute infections which produce changes in the blood picture resembling myelogenous leucemia, and in certain of such cases, even the necropsy findings are suggestive of leucemia.<sup>10</sup> Other infectious conditions reproduce a blood picture similar to that of lymphocytic leucemia. The most familiar examples of this are infectious mononucleosis<sup>11</sup> (glandular fever) and whooping cough. In other instances, tuberculosis may produce a leucemoid blood picture of the lymphocytic type. In one case observed personally, a child not only had the blood picture of lymphocytic leucemia, but also the clinical and laboratory findings of hemorrhagic purpura which so commonly occurs in the terminal stages of this form of leucemia. It was only at necropsy that a generalized miliary tuberculosis was demonstrated.

The infectious lesion which most closely resembles monocytic leucemia is Vincent's angina. This condition will frequently produce a mononucleosis with an increased percentage of monocytes or of abnormal mononuclear cells resembling monocytes. The differentiation presents an added difficulty since the organisms of Vincent's angina are almost invariably found on lesions of the mouth regardless of their etiology.

The clinical picture of monocytic leucemia is essentially that of any acute leucemia, as has been emphasized in the literature.<sup>2, 4, 12</sup> Infiltrative lesions of the gums and necrotic areas in the mouth form an especially prominent feature in most cases, and progressive weakness, fever or sore mouth are the most frequent symptoms of onset. On physical examination, enlargement of the lymph nodes may be slight or absent, but is never excessive, the spleen may or may not be enlarged to palpation and percussion, but at autopsy it is usually found to be moderately enlarged. Although an aleucemic form may exist, the leucocyte count is usually elevated and may reach very high levels. The differential count shows a high percentage of monocytes, many of which are of atypical form because of their immaturity. In practically all cases there are some myelocytes. A progressive anemia is a constant feature and a reduction of platelets with hemorrhagic tendencies is a common terminal event.

The origin of the monocyte is not definitely known and a clear conception of monocytic leucemia awaits a better understanding of this problem. A complete review of the subject will not be attempted here but only some of the conflicting opinions presented.

The theory that monocytes are derived from the myeloid series of cells in the bone marrow was held by Ehrlich<sup>13</sup> and by Naegeli<sup>14</sup> who would classify this type of leucemia as "monocytoid myelogenous leucemia." Piney<sup>15</sup> and Hittman<sup>16</sup> consider these cells to be of myeloid origin and believe that they can demonstrate definite transitional stages between monocytes and myelocytes. These authors hypothesize that monocytes develop from the reticuloendothelial system, but only through the intermediation of the myeloblast. Clough<sup>17</sup> stated

that the term 'monocytic leucemia' is applied to certain cases in which, at first, large numbers of monocytes appear in the blood. These cases have run the course of an acute myeloid leucemia, with the appearance later of many myeloblasts in the blood." However, he later reported a case of monocytic leucemia<sup>1</sup> and stated that recent studies yielded evidence that monocytes have an independent source of origin from the reticuloendothelial system.

Another view concerning the origin of monocytes is that they develop from the lymphocytic series of cells. Bloom<sup>18</sup> states that monocytes and lymphocytes cannot be differentiated by either the dry, supravital or oxidase stains. He believes that the monocytes develop from lymphocytes within the blood vessels where the flow is sluggish, and that he has traced transitional stages between the two forms. This view is also held by Pappenheim, Ferrata, Maximow, and Arneth.<sup>18</sup>

The belief that monocytes are of independent origin, has ardent supporters who adduce experimental evidence to support their theory. Schilling<sup>19</sup> could find no histologic or hematologic transition between monocytic and myelogenous leucemia and concluded that monocytes had an independent origin. Kellum and Forkner<sup>20</sup> present experimental data to show that the monocytes behave independently of the other series and do not proliferate with either the myeloid or lymphoid cells. Sabin,<sup>21-22</sup> with her coworkers and others who are working with the supravital stains, claim to be able to identify the monocyte by this means and assign to them an independent origin.

Tissue cultures have yielded nothing to support either theory,<sup>18</sup> since monocytes revert to macrophages in this procedure as do lymphocytes and other specialized cells under the same conditions.

From the evidence at hand the theory of the independent origin of the monocyte seems most tenable, although it has not been definitely proved. This impression must be subject to revision if conclusive proof is forthcoming to show that they originate from a common stem cell through the intermediation of either the myelocytic or lymphocytic series. While it offers no direct proof of their derivation as a separate cell type, the existence of a leucemia with a predominance of monocytes and without marked involvement of, or a transition to, either the myelocytic or lymphocytic series is of some significance.

#### SUMMARY

Three cases of monocytic leucemia are presented, two of which were studied by means of the supravital stains. The etiology, clinical course and diagnosis are reviewed briefly. The principal theories as to the origin of the monocyte are considered. In the light of our present knowledge, together with the fact that in certain cases of leucemia the monocytes proliferate independently of the other series, we believe it is justifiable to classify them as a separate series of leucocytic cells.

Since this manuscript was prepared additional cases have been reported by Farrar, G. E. and Cameron, J. D. *Am. J. M. Sc.* 184: 763, 1932 and Sydenstricker, V. P. and Primmy, F. B. *Am. J. M. Sc.* 184: 770, 1932.

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# LABORATORY METHODS

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## A SIMPLE ONE-STAGE TECHNIC FOR HEPATECTOMY IN THE DOG\*

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WITH SOME REMARKS ON THE CLINICAL SYMPTOMATOLOGY OF TERMINAL  
HEPATIC INSUFFICIENCY

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TWO methods are commonly used to excise the liver of laboratory animals. The classical method of hepatectomy devised by Mann (1921) of The Mayo Clinic has proved to be an eminently satisfactory procedure in the hands of those who have taken the trouble to learn it. This method is applicable only to dogs and consists of three separate operations, of which the first two are for the purpose of forcing a collateral circulation to develop for the portal vein and vena cava so that at a final operation the liver may be removed without disturbing the animal in any other way. The first stage of this method consists in a reverse Eck-fistula. Markowitz and Soskin (1927) modified Mann's principle for removing the liver by partially tying the portal vein and vena cava, so that the increased venous pressure caused a similar collateral circulation to develop. At a subsequent operation the liver could be removed, there being sufficient collateral circulation to permit an adequate venous return from the posterior half of the body. Mann and Graham (1928) have made some slight objections to this method on the grounds that it is not always certain that sufficient collateral vessels have developed at the time of the hepatectomy. Markowitz and Soskin were well aware of this but preferred to limit their method to a two stage operation, realizing that it could always be transformed into a three-stage operation, the second of which would consist in completely tying the partially ligated vessels. In actual practice the two-stage method has worked out very well, not only for dogs but for rabbits, monkeys, and guinea pigs. Diury (1929), for instance, employed a modified two stage method for removing the liver of rabbits with very satisfactory results, reporting liverless rabbits that lived as long as forty hours.

These methods for removing the liver of dogs entail one or more preliminary operations. It is no injustice to these methods to state that they are very troublesome and for the average physiologic laboratory are not applicable, since they entail the preparation and maintenance of a supply of animals

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for long periods of time when a research is being done requiring liverless dogs. We thought it would be of value to physiologic workers if a simple one-stage method were available that would permit complete removal of the liver without disturbing the animal in any other way. It is true various procedures are described in the literature for tying off the vessels to the liver after shunting the portal blood to the vena cava, but these methods leave a gangrenous organ in the abdominal cavity of which the toxicity, as Mann and his pupils, and Ellis and Dragstedt (1930), and others have shown, is enormous. In our opinion such experiments are rarely justifiable even apart from the fact that a certain amount of blood reaches the liver through the diaphragmatic veins and through the vena cava during the excursions of the diaphragm.

Frior and Stinson (1929) recently described a one-stage method for removing the liver of laboratory animals by cannulating the vena cava above the liver, the portal vein and the vena cava below the liver, and after this was completed, removing the liver by stripping from the cannula between these points. For this operation a Y-shaped cannula was used. The dogs lived from twelve to sixteen hours. Frior and Stinson were probably not dealing with animals that died of uncomplicated hepatic insufficiency. The operation of hepatectomy is in itself sufficiently disturbing to the animal without the added burden of complete obstruction to the portal vein and vena cava in the interval that these structures are being connected by a cannula. This method for removing the liver may be recommended to those who have not mastered the technic for doing an Eek-fistula. We believe the method described by us to be a superior one. The operation is easier, and the animals live longer and in better condition.

One of the great difficulties attending the final operation of hepatectomy by the multiple stage method has been hemorrhage during the final removal of the liver. This is due to the abundance of new anastomotic channels in the peritoneal adhesions. In the hands of the inexperienced this difficulty is generally an insuperable one and accounts for the relatively short survival period reported by most workers who have dehepatized dogs. One of the merits of a one-stage technic for hepatectomy is the absence of hemorrhage from this source.

It is one purpose of this paper to describe a thoroughly tried, simple method for excising the liver of a dog at one operation, which lasts about forty minutes. The operative mortality is slight, once the technic has been mastered. This method is based on the principle described by Frior and Stinson that a glass cannula of wide caliber may be inserted in the inferior vena cava without the development of any clotting whatsoever over a period of twenty-four hours. In the dog some hours following hepatectomy the clotting power of the blood becomes diminished, partly due to its lowered fibrinogen content. Consequently one may predict that the inferior vena cava traversing the liver may be reconstructed by a glass tube without signs of clotting in the tube. At first view this observation appears irreconcilable with the current knowledge of the clotting of blood. Perhaps it may be explained on the grounds that normal blood contains traces of heparin and antithrombin which, when

the minute volume of blood flow is great enough, will suffice to prevent coagulation over a smooth foreign surface, such as a glass cannula. The principle for our method of hepatectomy, as illustrated in Fig 1, is to tie a paraffined Pyrex glass cannula into the inferior vena cava, inserting it and tying it just below the liver. The glass cannula projects into the thoracic portion of the inferior vena cava. Blood from the hepatic veins can flow uninterruptedly around the paraffined glass surface. An Eck-fistula is then rapidly performed so that the portal stream is completely diverted to the vena cava below the cannula. The structures in the lesser omentum are next tied and the vena cava between the liver and the diaphragm is securely ligated over the cannula. The liver is then removed by several cautious cuts of the scalpel.

It might be suggested that the removal of the liver in one stage is too drastic a procedure. The subsequent behavior of such dehepatized dogs, however, compares favorably with the best prepared by the multiple-stage method. As a matter of fact, the preparations are usually in much better condition than the average animal dehepatized by the older methods.

We are proposing this method as a preferable one to the multiple-stage methods for 90 per cent of experiments requiring the use of liverless dogs. When the investigation concerns the coagulation of blood, it would be wise to accept with reserve conclusions drawn from animals dehepatized according to this method. Such experiments should be confirmed on preparations from which the liver has been removed after forcing the development of a collateral circulation for the portal vein and inferior vena cava. This is preferable, probably, to endothelializing the glass cannula by threading a strip of vena cava through it and reflecting it back over the ends.

#### TECHNIC OF THE OPERATION

The animal should be fasting for at least twenty four hours. This is important if a long lived preparation is wanted.

The first step in the operation consists in aseptically exposing the inferior vena cava just below the liver, ether anesthesia being employed. A midline incision is preferable, since it saves time and avoids some loss of blood from the incision which an S shaped incision always entails. After the insertion of a wide, self retaining retractor, the intestines are collected in the hollow of the duodenal mesentery. They are then wrapped in hot towels and packed off from the operative site as described by Fishback (1927) in his account of The Mayo Foundation technic for the establishment of an Eck fistula. The hepatorenal ligament is cut. The inferior vena cava is freed from its attachments to the posterior body wall between the lumbodrenal vein and the point where the vena cava becomes buried in the liver. Three ligatures are passed under the inferior vena cava, the middle one consisting of strong packing cord about 2 mm in diameter. The caudad ligature is a temporary one to prevent escape of blood during the cannulation. It is tied tight over a small brass rod as near the lumbodrenal vein as possible. With the left index finger under the vena cava and occluding it, a small cut is made with scissors in this vessel about 5 mm below the liver. A paraffined Pyrex glass cannula about 8 cm long and of suitable diameter is inserted into the vena cava through this opening, air embolism being prevented by the operator holding his thumb over the protruding end. The assistant meanwhile raises the flap of the vena cava by a mosquito forceps. The cannula is pushed into the vena cava as far as it will go, and the caudad end is then made to engage in the freed segment of the cava by pulling gently on a thread tied around the cannula about 10 cm from its lower end (see Fig 1). As soon as it engages, the previously placed ligatures are tied by the assistant on each side

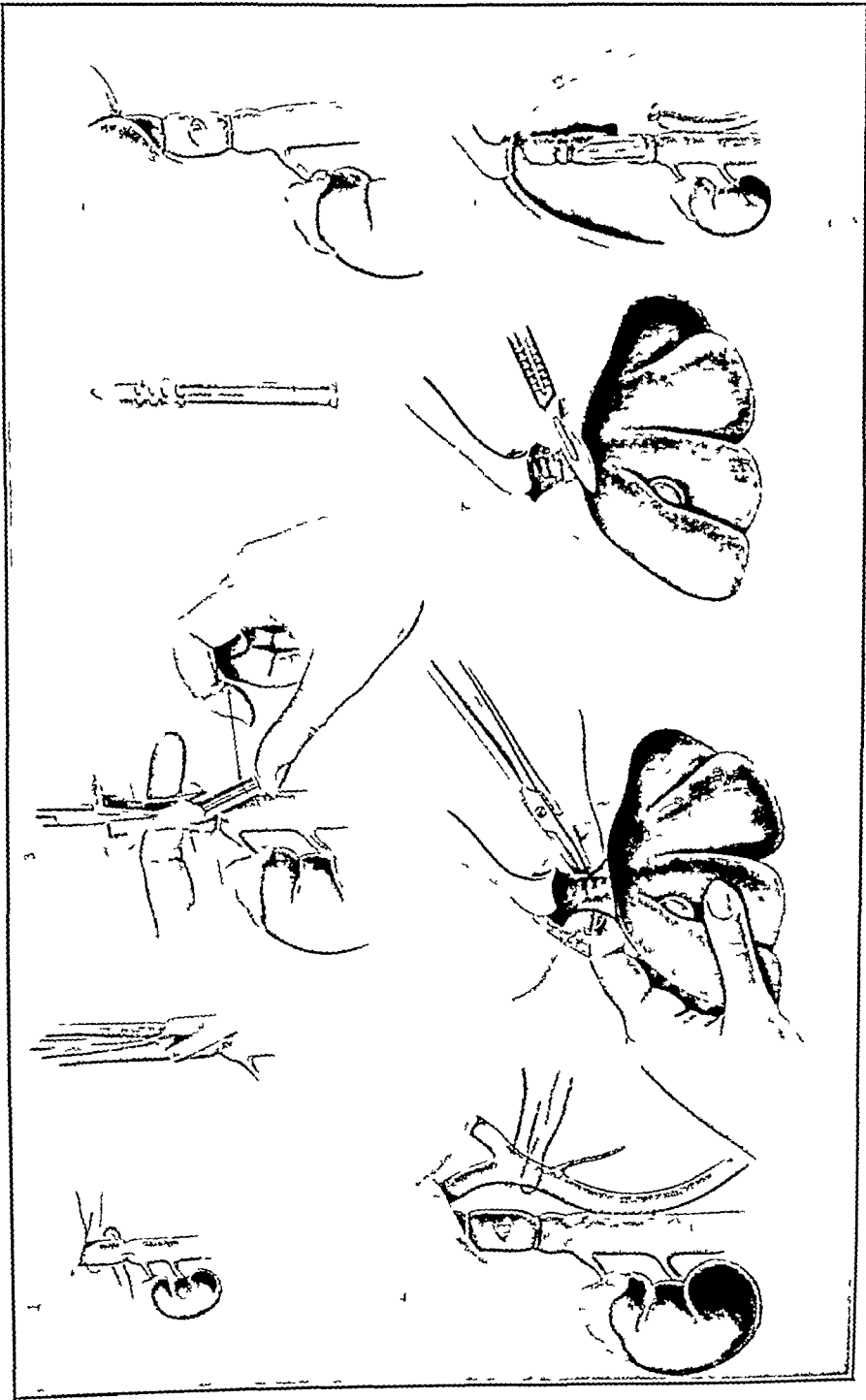


Fig. 1.—Steps in One-Stage Technique for Hepatectomy in the Dog. (Diagrammatic) 1, 2, 3, and 4 illustrate technique of isolating vena cava and inserting cannula (3d). 5 shows Lick-fistula completed. 6 and 7 show placing of ligatures on diaphragmatic area. 8 illustrates general appearance of diaphragmatic area after removal of the liver.



of the opening in the vessel. The ligature over the brass rod is now quickly cut, thus relieving the obstruction to the inferior vena cava. The complete obstruction of the vena cava need not last more than ninety seconds.

The second step of the operation consists in clearing away the tissue over the portal vein and establishing an Eck fistula. This is performed by the ingenious method of Fischler and Schroder (1909), as described by Fishback. The Eck fistula should be rather low (at least 1 cm. from the edge of the cannula) and the stoma should be as large as possible. To make an Eck fistula in a seven kilo dog is quite practicable, but animals smaller than this are not recommended. For a seven kilo dog the stoma should be about 2 cm. long. It is possible to complete the operation successfully with a smaller stoma, but we have always felt that in such cases there may be a certain amount of back pressure in the portal vein. In placing the suture lines it is advantageous not to sew into the tributaries of the portal vein at the caudad extremity of the stoma.

The third part of the operation consists of the hepatectomy proper. The portal vein is doubly tied at a safe distance above its last tributary (the pancreaticoduodenal vein) and cut between the ligatures and a Carmalt clamp. The three peritoneal folds binding the liver to the diaphragm are exposed and carefully cut. A packing cord ligature (2 mm. in diameter) is now placed around the vena cava between the diaphragm and the liver. This maneuver should be accomplished without exerting traction on the Eck fistula, by introducing the left hand into the right subdiaphragmatic region. The ligature follows the hand as it is withdrawn from this track. The operator pulls the liver gently in a caudad direction as his assistant ties the ligature as tightly as possible around the cannula. The ligature must be tied below the white margin of the diaphragm's junction with the cava. Three or four more ligatures are now tied to reinforce this one. The first loop of each tie is held taut by a mosquito forceps as the tie is completed. It is important in placing these ligatures to bear in mind the existence of the small lobe which is tucked away dorsally along the vena cava close to the esophagus.

The liver is now removed with several cautious sweeps of the knife. This removes the strip of vena cava over the cannula which is left bare. The last trace of liver tissue which sometimes adheres to one of the ligatures must be carefully removed.

There is often some bleeding from the diaphragmatic veins which have been torn as they course through the coronary ligaments. They should be picked up and tied. Bleeding does not occur from the Eck fistula, either at the time or the operation, or at any time thereafter.

We strongly recommend at this stage that one or two toy balloons be placed in the hepatic site. The balloons are inflated so that their volume is approximately that of the liver.

The fourth stage consists in sewing up the incision and placing the animal in a warm environment until recovery is complete. This is rapid and usually sthenic in nature. Usually the animals are surprisingly vigorous, walking about the room and displaying the normal curiosity concerning the objects in their environment.

#### POSTOPERATIVE BEHAVIOR

Such a preparation should live in good condition for about seventeen hours, if the nature of the experiment is such as not to injure the animal's vitality and providing glucose is given intravenously. It may live twenty or more hours, but this is unusual. For the first ten hours or so, hourly injections of one-quarter gram of glucose per kilogram should be given intravenously. Beyond this time the quantity of glucose necessary to maintain the blood sugar at a normal value has to be doubled, and if the animal lives over sixteen hours, it may have to be trebled, since hypoglycemic symptoms ultimately ensue at a higher blood sugar level than the clinical symptoms would suggest.

Ultimately, after a number of severe attacks of nausea and vomiting, the animal develops coma, which may be broken by remissions during which the dog gets up and walks about its cage. Pronounced tachycardia exists. There is an abnormal pinkness of all the visible mucous membranes, as though indicative of widespread vascular relaxation. The temperature is elevated. Soon the breathing becomes very noisy and may be irregular. Usually the pupil is much constricted. The combination of constricted pupils with flushed oral mucous membranes differentiates this condition of terminal hepatic insufficiency from gross internal hemorrhage. The arterial blood pressure at this time, as measured in one dog, was 65 mm mercury. The limbs show a peculiar rigidity with exaggerated tendon reflexes.

This type of coma may last several hours, or only a few minutes, before the animal gives a convulsive gasp or two and dies. Usually the heart beats for some time after respiration ceases.

At autopsy the fatty tissues of the body are jaundiced. There is always some bloody fluid in the peritoneal cavity which in well-prepared specimens, weighing about 12 to 13 kilograms, is usually about 150 cc, and which on centrifuging can be demonstrated to be about one-seventh erythrocytes. The visceral peritoneum shows extensive capillary hemorrhages. Usually the mucosa around the orifices of the pancreatic and biliary ducts is hemorrhagic, sometimes extremely so. This finding is more marked when the stoma of the Eck-fistula is undersized, but may be present to a marked degree when it seems as though the stoma is more than ample. The hemorrhagic state of the visceral peritoneum is very characteristic and is more obvious where this membrane has been abraded by indelicate handling during the hepatectomy. The autopsy is otherwise negative except for the occurrence of hemorrhages in various organs, which are especially constant in the subendocardial surface of the ventricles. The glass cannula is free of clot even when some of the shed blood contains enough fibrinogen to clot quickly in a test tube.

If, after the hepatectomy the animal has been gently catheterized, subsequent specimens of urine will contain blood. This is constant even when a soft rubber catheter is used. It is an expression of the hemorrhagic tendency which is so prominent a feature of severe hepatic insufficiency. At autopsy hemorrhagic areas in the bladder mucosa will be noted.

Certain clinical features presented by a liverless dog may now be mentioned. At first the flow of urine is normal. Usually after the eighth hour the quantity of urine obtainable is scanty, even when large amounts of fluid are given intravenously. We have a feeling that this oliguria is partly related to the washing out of urea, of which the formation, as The Mayo Foundation studies have shown, is in abeyance after the hepatectomy. It may, of course, be due to a lowered arterial blood pressure in consequence of a gradual arteriolar relaxation. At any rate, large quantities of urine (50 to 75 cc each hour) are obtainable if the animal (in the oliguric stage) is given hourly 100 mg urea per kilogram and 1 gm of sodium sulphate per kilogram, both dissolved in about 75 cc of water.

One of the striking and constant features about a liverless dog is the tachycardia. Immediately after hepatectomy the pulse rate is high until the

anesthetic has been largely blown off. Ninety minutes after hepatectomy in a 13 kilogram male bull terrier it was 124 (with the animal resting on its side). It hovered around this figure for three hours. Four hours after hepatectomy it was 165, around which figure it hovered for an hour or so. Seven hours after hepatectomy it was around 180. An hour later it was around 200, and by twelve hours after removal of the liver it was 280 per minute. Fourteen hours after hepatectomy it was around 260 per minute. An hour later it was 300 per minute. Ten minutes before death (twenty hours after hepatectomy), the heart rate was 260 per minute.

These facts indicate that the tachycardia being of gradual onset, is not the immediate result of the intraabdominal surgery. There is reason to believe that it is not due to an oversecretion of adrenalin, for such quantities of adrenalin would cause dilatation of the pupil and pallor of the visible mucous membranes. Moreover, these facts were manifested by an adrenalectomized liverless dog.

Roughly speaking, the intensity of the tachycardia is proportional to the severity of the animal's general condition. It is conceivable that it is reflex, dependent simply on a low blood pressure. The tachycardia of simple hypotension, however, is rarely as marked as that of hepatic insufficiency. Also, we have positive evidence that the rapid pulse rate is independent of the nervous system, and is a direct result of metabolic changes ensuing in the liverless animal. This feature will be discussed in a future paper. We are here drawing attention to its great constancy, and to its approximate value as a criterion of the extent of the hepatic insufficiency.

Vomiting is another characteristic symptom of severe hepatic insufficiency in the liverless dog. Sometimes it is severe and intractable, the animal standing in the corner of its cage and retching steadily for several minutes about four times an hour. In our experience, vomiting always occurs if the animal lives long enough, and severe retching may occur intermittently for five hours. The act may exhaust the dog. Thus one of our dogs retched violently, and was dead five minutes later. We suspected in this instance that a diaphragmatic ligature had slipped, allowing bleeding from the cut vena cava, but the autopsy was negative.

The other symptoms of severe hepatic insufficiency, such as jaundice, hypoglycemia, etc., have been adequately commented on by Mann and Magath. The terminal picture of hepatic insufficiency presents the following characteristic symptoms: Tachycardia (with hypotension), jaundice, severe vomiting, hemorrhagic diathesis, oliguria, and (as Mann and others have shown) certain characteristic changes in the composition of the blood, indicative of the rôle played by the liver in uriccolysis, deamination and urea formation, the synthesis of blood fibrinogen and of blood sugar. These changes in aggregate undoubtedly tax the physiologic adjustments of the animal to the utmost, and the question may be asked whether the terminal coma is dependent on the combined trauma to the organism inflicted by these changes or whether "second-stage" hepatic insufficiency (Mann) is due to a specific abnormality, comparable to the hypoglycemia which is undoubtedly the cause of "first-stage" hepatic insufficiency. Frior and Stinson stated that the ultimate cause of

death following hepatectomy is not clear. They ventured that it might be due to progressive heart failure, preceded by vomiting. The question must be left unsettled for the present. The terminal symptoms are certainly not due to absence of glutathione from the blood (as Cahoon has shown in this laboratory).

We have encountered the supposedly characteristic rigidity and noisy, irregular breathing in a comatose dog, five hours after hepatectomy, when the autopsy disclosed that death was due to a slipped diaphragmatic ligature, with severe hemorrhage. McMaster and Drury (1929) failed, many hours after hepatectomy, to benefit liverless rabbits by connecting them to normal rabbits by means of a cross circulation, which is good evidence that the terminal symptomatology is not due to the absence of a chemical from the blood or tissues. They look upon the cause of death as an irreversible change, resulting from a perverted metabolism consequent upon removal of the liver. However, the question of whether the ultimate cause of death following hepatectomy is due to a deficiency of a chemical, or to a surplus of one or more noxious chemicals, must be left undecided for the present.

#### SUMMARY

A one stage technic for excising the liver of dogs is described.

The symptomatology of dogs with terminal hepatic insufficiency is described.

At our request, Dr. F. C. Mann kindly investigated the feasibility of our method. He reported that it was feasible and satisfactory in his hands.

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## THE DETERMINATION OF NONPROTEIN NITROGEN WITH SPECIAL REFERENCE TO THE KOCH-McMEEKIN METHOD\*†

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THE determination of nonprotein nitrogen by the Folin method<sup>1</sup> is, perhaps, the most difficult of the routine analyses made on blood. Inexperienced technicians, especially, have a great deal of trouble in obtaining clear solutions free from particles of silica. When Folin's original instructions are followed, it is almost impossible to avoid the separation of silica from the glass during digestion. This is the result of heating the digestion mixture for too long a period. However, if the acid mixture is heated rapidly until it has a light green color there is much less danger of the glass being attacked.

We have found that the best results are obtained by quickly boiling down the solution with a high flame until the ignition tube is completely filled with dense white fumes of sulphur trioxide. Then the flame is partly reduced so that the digestion mixture still boils briskly. The heating is continued just to the point where the material has a light green appearance. The exact depth of color can only be determined by experience. It is essential to use a good quality quartz pebble to prevent "bumping," since the ordinary grade of glass beads is readily attacked by the phosphoric acid in the digestion mixture.

Although this technique eliminates turbidity due to silica it is open to several objections. In the first place there is the possibility that the oxidation will not be complete in the shorter digestion period. Also, close attention and careful regulation of the flame are essential. For this reason it is difficult for one individual to run more than two or three determinations at a time. Even though adequate care is used, an occasional determination will require repeating or centrifuging because of the action of the digestion mixture on the glass.

If the solution is nesslerized while turbid, there is an increased tendency for the nesslerized solution to become "cloudy" due to a slight precipitation of the reagent. When this occurs, after centrifuging the top of the white layer of silica precipitate will appear red. If this happens the determination must be rejected because the results will be low. On the other hand when the nesslerized solution is read in the colorimeter while turbid, either from a precipitation of the reagent or from silica, the results will be too high. The practice of many technicians in reading nesslerized solutions which are not entirely clear is likely to confuse the physician in "borderline" cases of nitrogen retention.

Because of the disadvantages inherent in the Folin-Wu<sup>1</sup> procedure, many other methods and modifications have been proposed. The procedures of Koch-

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McMeekin<sup>2</sup> and Wong<sup>3</sup> seem to be the most practical of these methods. Koch uses hydrogen peroxide for the oxidizing agent, while Wong employs potassium persulphate. Since both of the methods use only sulphuric acid for the digestion mixture, there is no danger of detaching silica from the digestion tubes.

However, when sulphuric acid with peroxide or persulphate is used for the digesting agent, a precipitate of tungstic acid nearly always appears, either during digestion or after water is added. The color of the particles of tungstic acid may be white, gray, yellow, or yellowish green. These compounds, as emphasized by Morley,<sup>4</sup> are very soluble in sodium hydroxide. Therefore the turbidity due to tungstic acid disappears when the solution is nesslerized.

Although the alkaline Nessler's solution readily dissolves the tungstic acid, the reagent itself is affected in the process, so that the nesslerized solution is usually somewhat turbid. Therefore the methods of Koch and Wong have a very limited value in the determination of nonprotein nitrogen when the filtrate contains significant amounts of sodium tungstate. However, with trichloroacetic acid filtrates the above methods give excellent results.

As a matter of fact when only nonprotein nitrogen determinations are to be made trichloroacetic acid is preferable to tungstic acid for removing the proteins. The solution filters faster and a larger yield of filtrate is obtained. Hiller and Van Slyke<sup>5</sup> have shown that a 25 per cent trichloroacetic acid precipitating mixture gives the same values for nonprotein nitrogen as tungstic acid.

We studied the problems involving tungstic acid precipitation and silica formation by using the Folin<sup>1</sup> tungstate precipitating solution with a standard nitrogen solution instead of blood. In this way the maximum effect of the tungstic acid was produced since the precipitating blood proteins remove varying amounts of the tungstic acid. We found that sulphuric acid had little power to hold tungstic acid in solution. On the other hand phosphoric acid proved very effective in preventing the precipitation of tungstic acid.

It was thought that the advantages of both the Koch and Folin methods could be combined by using peroxide for the oxidizing agent and just enough phosphoric acid to keep the tungstic acid in solution, yet not sufficient to attack the glass. This combination did not prove entirely satisfactory because the new digestion mixture often removed extremely fine particles from the ignition tube, even though small amounts of phosphoric acid were present.

Folin<sup>6</sup> recently reported that gum ghatti was very efficient in stabilizing colloidal solutions. Later Looney<sup>7</sup> and Folin<sup>8</sup> found that the gum was a very satisfactory protective agent in the direct nesslerization of nitrogen solutions.

We have worked out a modified Koch method in which gum ghatti and sodium citrate are used to prevent the precipitation of the color reagent when solutions turbid from tungstic acid are nesslerized. Hubbard<sup>9</sup> uses Rochelle salt to prevent the precipitation of Nessler's solution but we find that sodium citrate is somewhat more efficient in our modified method. However, neither salt has very much protective power when used alone. As a matter of fact, nesslerized standard solutions of nitrogen do not seem to be quite as clear when either of the salts is present. Nevertheless, we believe that sodium citrate and gum ghatti together give better results than when the gum is used alone. In

order to simplify the procedure when many determinations are being made, the digestion mixture is made up with hydrogen peroxide. This peroxide sulphuric acid solution will remain serviceable for at least a week at room temperature under ordinary laboratory conditions.

The instability of the digestion mixture is not a serious disadvantage since a weak solution can be readily made stronger by adding a little more peroxide and sulphuric acid. However, if only an occasional determination is made, it is not worth while to make up the digestion mixture containing the oxidizing agent. The acid mixture No. 1 is used and the material oxidized during digestion, without removing from the flame, by adding a drop of 30 per cent hydrogen peroxide as soon as the contents of the tube become brown, just before the white fumes appear.

We have found, as have others, that Koch's Nessler's solution is more satisfactory than Folin's. Koch<sup>2</sup> used 12 cc of his reagent for a determination when the digestion mixture contained 50 per cent concentrated sulphuric acid. However, we prefer to use a digestion mixture containing 45 per cent sulphuric acid and to dilute the Nessler's solution so that 15 cc will give the same final alkalinity as 12 cc of the original reagent. This dilute Nessler's solution can also be used in the regular Folin method.

In the Folin procedure the Nessler's solution is usually measured in a cylinder and quickly added to the 35 cc of nitrogen solution in the ignition tube. Then the tube is stoppered and the solution is mixed. The results are usually satisfactory when this procedure is carried out quickly. The density of the Nessler's solution is enough to cause, when quickly added, sufficient mixing to prevent a precipitation of the reagent. We prefer to nesslerize by pouring the reagent, and the sample diluted to 35 cc, simultaneously into a large beaker and then pouring back into the tube. In this way the two solutions are uniformly mixed and there is less danger of turbidity. Also it saves the extra step of stoppering and inverting the tubes in order to mix. Ordinarily the same beaker may be used for all of the nesslerizations without introducing significant error. It is necessary to employ this technique when using the dilute Koch reagent since its density is insufficient to obtain satisfactory results when the nesslerization is made directly in the tube.

#### REAGENTS

A stock solution of sulphuric acid is prepared by adding 225 cc of C P concentrated sulphuric acid to 245 cc of distilled water in a liter flask. The acid should be added a little at a time and the solution cooled by rotating the flask in a stream of cold water. From this stock solution of sulphuric acid, two acid mixtures are prepared.

Digestion mixture No. 1 is made up by adding 6 cc of water to 94 cc of the stock sulphuric acid solution. This acid mixture does not contain peroxide and is used for making up the standard, and also when the oxidizing agent is added separately to the material being oxidized.

Digestion mixture No. 2 is prepared by adding 3 cc of 30 per cent hydrogen peroxide to 47 cc of the stock sulphuric acid solution. It more convenient, 3 per cent peroxide may be used. In this case 45 cc of ice cold C P

concentrated sulphuric acid is slowly added to 55 c.c. of ice cold 3 per cent hydrogen peroxide. Acid mixture No. 2, containing peroxide, is used for digesting the sample.

Before using, it is advisable to titrate the two acid mixtures (peroxide does not interfere with the titration) with the modified Koch Nessler's solution. One cubic centimeter of the mixture requires from 10.1 to 10.7 c.c. of the Nessler's reagent, phenolphthalein being used as an indicator. Also, the amount of peroxide in digestion mixture No. 2 can easily be checked. One cubic centimeter of the peroxide mixture should decolorize about 9.5 c.c. of 0.1 N potassium permanganate. When the digestion mixture decreases in strength until less than 4.5 c.c. of potassium permanganate is used up, more peroxide and sulphuric acid must be added in the proportion, 1 c.c. of 30 per cent hydrogen peroxide and 0.8 c.c. of concentrated sulphuric acid to 25 c.c. of weak digestion mixture.

For the standard nitrogen solution weigh out 0.236 gm. of ammonium sulphate or 0.1911 gm. of ammonium chloride, dissolve in a little water and dilute to 1000 c.c. Three cubic centimeters of this solution are equivalent to 0.15 mg. of nitrogen.

The 1 per cent solution of gum ghatti is made by selecting 1 gm. of the gum tree from dirt and suspending it in a piece of gauze at the top of 100 c.c. of water in a cylinder. After standing for ten or twelve hours the bulky residue in the bag is discarded and the clear gum solution used as it is. Best results are obtained when the gum is kept at room temperature without preservatives and made up fresh each week.

For the modified Koch Nessler's solution, dissolve 22.5 gm. of iodine in a liter Florence flask containing 20 c.c. of water and 30 gm. of potassium iodide. When the material has dissolved, remove 0.5 c.c. of the iodine solution and save for adjusting the reagent later in the procedure. Now add 30 gm. of pure metallic mercury to the solution in the flask and rotate the contents rapidly, keeping the mixture from becoming hot by holding under a stream of cold water from time to time. Continue shaking the flask until the supernatant liquid has lost practically all of the reddish yellow color due to iodine. Allow the particles to settle for a few minutes, then carefully decant the supernatant liquid and test for the presence of iodine by adding a few drops to a 1 per cent starch solution on a spot plate. Unless a positive test is obtained the solution may contain mercurous compounds. If the test is negative, add a few drops at a time of an iodine solution, using the 0.5 c.c. which was set aside, until a very faint test for iodine is obtained. Dilute at once to 200 c.c. and mix. To 97.5 c.c. of an accurately prepared 10 per cent sodium hydroxide solution add the entire amount of potassium mercuric iodide solution prepared above. Now add 27 c.c. of 10 per cent sodium citrate solution and dilute to 1600 c.c. Mix thoroughly and allow to clear by standing.

If this Nessler's solution is to be used instead of Folin's reagent in the regular Folin method, the sodium citrate is omitted. The alkalinity of the Nessler's solution should be checked by titrating against 20 c.c. of normal hydrochloric acid, using phenolphthalein as indicator. From 12 to 13 c.c. of the reagent will be required to neutralize the acid if the alkalinity is satisfactory.



# PROCEDURE

To 5 cc of blood filtrate in a Pyrex ignition tube graduated at 35 and 50 cc, add 1 cc of the sulphuric acid-peroxide digestion mixture No 2 and two glass beads or a quartz pebble. Heat with the full flame of the micro burner until the dense white fumes of sulphur trioxide appear. Close the mouth of the tube with a small funnel and regulate the flame until the digestion mixture barely boils. At this point the material should be completely oxidized and the digestion mixture will be colorless. However, the presence of a tungstic acid precipitate will give a white, yellow or yellow green appearance to the contents of the tube. If there is a definite brown color due to unoxidized material, the digestion mixture is too weak and more peroxide must be added. Continue the very gentle heating for about two minutes, then remove from the flame. There are usually significant amounts of peroxide present at the end of the digestion but they do not interfere when the solution is nesslerized. When occasional determinations are made it is more convenient to use digestion mixture No 1 and add the oxidizing agent separately. Either 1 drop of 30 per cent or 10 drops of 3 per cent hydrogen peroxide or 15 drops of a saturated solution of potassium persulphate may be used. In either case the oxidizing agent is added during digestion, just when the contents of the tube become brown. It is not necessary to remove the tube from the flame when adding the peroxide in this way since the digestion mixture still contains enough water to prevent spattering. The small funnel is then placed in position and the digestion completed as described above.

When the digested material is cold, rinse the funnel, both inside and out, add 1 cc of 1 per cent gum ghatti and dilute to 35 cc. In another tube place 1 cc of the digestion mixture without peroxide, No 1, 3 cc of the standard nitrogen solution and 1 cc of gum ghatti and dilute to 35 cc. Measure out 15 cc of the Nessler's solution with sodium citrate in a small cylinder. Now quickly pour the Nessler's reagent and the 35 cc of nitrogen solution simultaneously into a 400 cc beaker, whirl and pour back into the graduated combustion tube. (It is important for the solutions to be at room temperature when nesslerized since increased temperature encourages precipitation of the reagent.) In a minute or two after the air bubbles have disappeared the nesslerized solutions should be crystal clear. The standard and unknown solutions are nesslerized as near together as possible and at once read in the colorimeter. Calculation of results is made in the regular way using tables or the general formula

$$\frac{\text{Reading of S}}{\text{Reading of U}} \times \frac{\text{Vol of U}}{\text{Vol of S}} \times \frac{\text{mg N in cc of S used}}{\text{cc of U used}} \times 100 = \text{mg nonprotein nitrogen per 100 cc of blood}$$

Setting the standard at 20 mm and using 3 cc of the standard nitrogen solution and 0.5 cc of blood, the above formula reduces to

$$\frac{600}{\text{Reading of U}} = \text{mg N per 100 cc of blood}$$

This modified Koch method has been checked against the Folin procedure and satisfactory agreement obtained. Most of the data given in Table I were obtained from single analyses but when check determinations were necessary the new method nearly always gave better agreement than Folin's. For highly accurate results the nitrogen in the reagents should be determined by using a urea solution (made up with 0.5 per cent glucose) of known nitrogen content, in place of the blood filtrate.

TABLE I  
COMPARISON BETWEEN THE MODIFIED KOCH AND FOLIN'S METHOD  
Mg Nonprotein Nitrogen per 100 cc Blood

NO	PATIENT	DIAGNOSIS	KOCH	FOLIN	DIFFERENCE	DIFFERENCE
			mg	mg	mg	per cent
1	C G	Nephritis	42.0	43.5	-1.5	-3.5
2	G R	Nephritis	36.2	36.8	-0.6	-1.6
3	F B	Nephritis	93.5	93.9	-0.4	-0.4
4	A P	Nephritis	34.5	37.3	-2.8	-7.5
5	H F	Nephritis	53.6	56.0	-2.4	-4.3
6	G P	Nephritis	69.0	68.2	+0.8	+1.2
7	F D	Diabetes	36.4	37.5	-1.1	-2.9
8	I G	Diabetes	30.0	30.3	-0.3	-0.9
9	M V	Diabetes	36.8	37.9	-1.1	-2.9
10	E F	Diabetes	42.3	40.6	+1.7	+4.2
11	E H	Hypertension	64.5	61.2	+3.3	+5.4
12	M H	Surgical	35.3	34.5	+0.8	+2.3
13	Mixed	Hospital blood	63.2	64.5	-1.3	-2.0
14	Mixed	Hospital blood	29.0	28.1	+0.9	+3.2
15	Mixed	Hospital blood	30.8	31.9	-1.1	-3.4

#### SUMMARY

The period of digestion in Folin's original directions is excessive for practical purposes. Best results are obtained by stopping the digestion as soon as the brown appearance of the digestion mixture is replaced by a light green color.

When sulphuric acid with peroxide or persulphate is used for the digestion mixture a white or yellow precipitate of tungstic acid nearly always appears either during digestion or when water is added. This acid precipitate is soluble in the alkaline Nessler's solution but the reagent itself is affected during the process of solution.

A practical modified Koch method for blood nonprotein nitrogen is described. The new method was checked against the Folin procedure and found to be accurate. This method gives promise of being the most satisfactory one available for routine use in the clinical laboratory.

The author takes pleasure in acknowledging his obligation to Dr Arthur Knudson for assistance with the manuscript, and also to Miss Mary L. Blackmer for trying out the method in the Albany Hospital Laboratory.

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### A MICRO-KJELDAHL STILL<sup>3</sup>

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WHILE various procedures have been proposed for the direct nesslerization of Kjeldahl digests in the determination of nonprotein and protein nitrogen, preliminary distillation of the digests continues to be favored by many workers. For such distillations the still described herein has proved, through several years' experience in this laboratory not only in students' use but in more exacting requirements, exceptionally effective and convenient.

The various parts of the apparatus (Fig 1) may be assembled readily in any laboratory. An iron stand (No C) with heavy tripod base supports the entire apparatus. The iron rod is slightly loosened in the base so that all parts attached to the rod may be rotated simultaneously without altering their relative positions. Receiving vessel and micro-burner are supported upon adjustable wooden platforms. The condenser† has a straight inner tube of Pyrex glass of such diameter as will be admitted by a 50 cc volumetric flask, the jacket may be cut from a 100 cc graduated cylinder or other tubing large enough to carry 2-hole rubber stoppers with water inlet and outflow tubes. A trap between condenser and distilling tube is made from a discarded 25 cc pipette. Burner and condenser tubings may be passed through the crevices between the iron rod and the several attached clamps.

With the still rotated to the right there must be sufficient clearance between the table and the end of the condenser tube to admit the receiving vessel. The latter is now raised and the still is rotated to the left until the vessel sits upon the wooden platform at such level that the tip of the condenser tube dips into the receiving acid.

Where the amount of nitrogen for digestion does not require more than 1 to 2 cc of  $H_2SO_4$ , the digestion is carried out in 25 x 200 mm Pyrex test tubes on a microdigestion rack. As much as 30 or 40 cc of fluid may be boiled down safely and without attention in such a tube if a pinch of talcum powder is

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†A West type condenser is to be preferred.

added. When the residue begins to char a small funnel with sealed stem is placed in the mouth of the tube, this prolongs the time required for clearing but avoids loss by spattering. When digestion is complete, the funnel is washed down by 10 to 15 cc of water, a little more talcum is added, and sufficient saturated NaOH. The total volume for distillation should not be more than 20 to 25 cc. The tube is attached to the still, shaken to mix the contents, and clamped in position. Heating is begun and continued at the top of the liquid

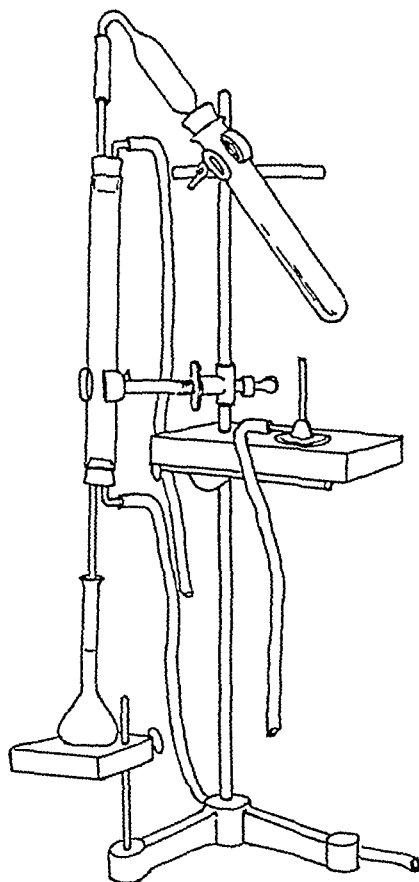


FIG. 1

column, the burner being moved back toward the lower end of the tube as distillation proceeds. Because of the small total volume displacement of the entire system it is rarely necessary to distill over more than 10 cc to assure complete transfer of such quantities of nitrogen as should be employed. Before the bumping stage is reached in the distillation the still is rotated to the right so that the receiving vessel may sit upon the table and the condenser tube be out of contact with the receiving fluid. After some further distillation in this position the condenser tube is washed down. By removing the flame some of the wash water is drawn well up into the condenser tube and then expelled by a momentary reheating of the distillation tube.

The transferred ammonia may, of course, be estimated either colorimetrically or titrimetrically. We commonly employ this still for quantities of nitrogen ranging between 0.1 and 2.0 mg, and it is our experience that the step of transfer alone of such quantities does not introduce into the final value a measurable error.

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## A STUDY OF THE KOLMER, KAHN, AND KLINE TESTS WITH SPINAL FLUIDS\*

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IN 1931 Kline and Rein<sup>1</sup> published a microscopic slide precipitation test for spinal fluid with 1006 specimens, comparing it with a somewhat lesser number of specimens, using the Mt. Sinai Hospital Wassermann reaction and an outside Wassermann test. They concluded that the slide test was somewhat more sensitive than the Wassermann test with the same antigen, and had the advantage of being much simpler in that it required no titrated hemolytic system. It had the advantage over the Kahn of not requiring a concentration of globulins. In 1932 Eller<sup>2</sup> and Rein compared the Kline microscopic precipitation test on 500 spinal fluids with the Wassermann test (ice box fixation), the Ishii modified Kahn test, the albumin globulin ratio, and the colloidal gold reaction. They concluded that the slide test was more sensitive than any of these tests and gave results which agreed with clinical findings more frequently than did the other reactions.

The purpose of the present study was to compare the Kline and Kahn precipitation tests with the Kolmer-Wassermann in order to ascertain which one of the precipitation tests might be used to greater advantage as a routine test with the Kolmer-Wassermann reaction in spinal fluids. The Kahn, as mentioned earlier, has the disadvantage of the time-consuming process of concentration of globulins and of requiring much more spinal fluid than does the Kline. However, the problem immediately presented itself regarding the Kline test, as to whether this reaction would prove practical in a State Laboratory where all the specimens are shipped in, because of the fact that according to Kline the test cannot be carried out on fluids giving a negative reaction for sugar. It was soon evident that many of the specimens received in our Laboratories were negative for sugar as indicated by the Benedict test. The question of the disappearance of sugar from spinal fluid is apparently still controversial. Levinson<sup>3</sup> states that "the decrease in the cerebrospinal fluid sugar is probably due to the lytic action of the cells and organisms present (P188)." He also states "Many normal fluids do not contain sufficient sugar to be reduced by Fehling's or Benedict's solution (P258)." Chevassut,<sup>4</sup>

\*From the Minnesota Department of Health, Division of Preventable Diseases.  
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in a series of more than 200 spinal fluids, showed the fluids contained a specific glycolytic ferment, and that the sugar disappeared in a few days even when the fluid was kept under sterile conditions, but this change was accelerated most at 37° and was inhibited at 0° C. On the other hand, Nielson<sup>7</sup> believes that the glycolytic process is due entirely to exogenous factors. He found glycolysis to vary greatly in spinal fluids, even sterile ones, in some beginning one hour after removal, and others not for thirty-two days. Staphylococci growth in the fluid did not influence the sugar content, but glucose fermenting organisms did. Furthermore, he found that leucocytes or temperature did not influence glycolysis.

In our series we have grouped the specimens under sugar reaction (1) positive, (2) negative, or (3) insufficient material to carry out the sugar test. The specimens classified under treated syphilis are from syphilitic patients in whom central nervous system syphilis had been definitely diagnosed, but includes some in which it was merely suspected. The untreated ones include specimens from patients in whom systemic syphilis had been diagnosed and previously treated, central nervous system syphilis now being suspected. Those unclassified include specimens in which no definite history could be obtained. It is regretted that more complete histories could not be procured to permit of a more detailed description. It will be noted by referring to Tables I and II, that while 1632 tests were carried out with the Kolmer and Kline, there were only 695 of these specimens in which the Kahn test was done.

#### TECHNIC

The technic as published by Kline<sup>1</sup> for spinal fluids was followed with the exception that the antigen was made according to his 1931<sup>2</sup> modification. We have found it necessary to titrate the Kline antigen, as different lots may vary considerably. New antigen was standardized to conform with the former lot of our antigen used, and with antigen furnished us by Kline. The standardization was accomplished by diluting the antigen with varying amounts of cholesterolized alcohol until uniform results were obtained, using the various antigens with at least 100 different sera of varying degrees of positivity as well as negative ones. Kline's technic was also modified in that spinal fluids giving negative reactions for sugar were used in the test. A large majority of these fluids were clear, only very rarely were there heavily contaminated ones. Fluids were also tested in which there was insufficient material to carry out the Benedict test for sugar. The Kolmer<sup>3</sup> technic was strictly followed. The Kahn<sup>4</sup> technic was used as given by him in 1929. All tests were read by two serologists and there was little variation in the readings. In this study we have considered 2+ to 4+ as positive in any test, although Kolmer considers a + as positive. Kline's method of reading his test is as follows: "± to 4+ any test in syphilitic serum equals agreement," which implies that a ± in a syphilitic serum is considered positive. He further states that "2+ to 4+ any test in nonsyphilitic serum equals false positive."

#### RESULTS

In Table I is shown the agreement and disagreement of the Kolmer-Kline, Kolmer-Kahn, and Kahn-Kline tests respectively, according to the sugar

TABLE I  
SHOWING AGREEMENT OF KOLMER, KAHN, AND KLINE

	SIGNATURE	TREATED SYPHILIS (C N S)		UNTREATED SYPHILIS		NO HISTORY OF SYPHILIS		UNCLASSIFIED		ALL GROUPS	
		TOTAL SIFC	AGREEMENT SIFC %	TOTAL SIFC	AGREEMENT SIFC %	TOTAL SIFC	AGREEMENT SIFC %	TOTAL SIFC	AGREEMENT SIFC %	TOTAL SIFC	AGREEMENT SIFC %
Kolmer-Kline	-	35	28 80.6	38	35 92.0	163	151 91.1	209	281 91.0	515	498 93.0
	+	55	52 94.5	68	61 89.7	312	310 99.3	379	340 91.7	811	763 93.6
	0	37	30 81.0	11	9 81.8	87	83 95.1	118	115 91.2	254	257 91.0
Total		127	110 86.6	117	105 89.7	562	547 97.3	826	776 91.9	1642	1515 91.0
Kolmer-Kahn	-	11	13 92.8	22	20 90.9	81	81 100.0	83	75 90.3	200	189 91.5
	+	10	26 86.6	46	39 82.6	208	208 100.0	171	155 89.0	478	427 87.2
	0	1		2	1*	19	18 91.7	15	8*	37	27 71.0
Total		15	39 86.6	70	60 85.7	308	307 99.6	272	238 87.5	695	613 92.5
Kahn-Kline	-	11	14 92.8	22	20 90.9	81	81 100.0	83	76 90.0	200	190 91.5
	+	30	27 87.0	16	41 93.0	208	207 99.5	171	151 88.0	478	429 87.0
	0	1		2		19	19 100.0	15	11 76.1	31	12 37.0
Total		15	30 86.6	70	61 87.1	308	307 99.6	272	213 78.3	695	651 91.6
All three	-	14	10 71.1	22	20 90.9	81	81 100.0	83	75 90.3	200	186 90.1
	+	30	26 86.6	16	39 82.6	208	207 99.5	171	151 88.0	478	426 87.0
	0	1		2		19	18 91.7	15	8*	37	26 70.0
Total		15	36 80.0	70	59 81.2	305	306 99.6	272	237 87.1	695	635 91.5

2+ to 4+ considered positive in all cases

Specimens noted as negative are comprised of the following:

All three tests negative - all three tests 2+, all three tests 2+ to 4+

Percentages are computed according to the agreement of specimens with serum reaction positive or negative according to Benedict or Insulin test material to carry out the serum test. The final percentage in each classification is compiled for the entire group regardless of sub-<sup>1</sup>

\*Too small for single group compilation but included in totals

<sup>1</sup>Includes only 635 specimens from Kolmer-Kline group

reaction, and in those in which there was not sufficient sugar to run the Benedict test. Although the percentage of agreement is variable for the several groups, the figures demonstrate no appreciable difference in agreement for specimens with or without sugar, respectively. In the total agreement the Kahn-Kline agreement is slightly higher (93.6 per cent) than in the Kolmer-Kline (93.0 per cent) or the Kolmer-Kahn (92.6 per cent). The figures are but a little higher than that of Kline<sup>1</sup> for his slide test and the Mt Sinai Hospital Wassermann test (92.3 per cent) in which all specimens contained sugar. The agreement obtained by Eller and Rem<sup>2</sup> with the diagnostic Kline test and the noncholesterinized Wassermann test in a combined group of proved neurosyphilitics and nonneurosyphilitics was 93.0 per cent, while their agreement in patients with no evidence of neurosyphilis was 99.59 per cent as compared to 97.3 per cent Kolmer-Kline agreement in our series in patients with no evidence of syphilis. Table I shows the percentage of agreement of the Kolmer, Kahn, and Kline with respect to positive and negative sugar reactions. This Table demonstrates that the agreement between tests is very close, irrespective of sugar content. The discrepancies which occur might possibly be explained by the difference in numbers of specimens in the two groups based on the Benedict test. Statistical calculation has demonstrated no significant difference in any of these figures. Furthermore, statistical calculation has shown no significant differences in results of the Kline test with and without sugar, or the Kolmer and Kahn tests with and without sugar, respectively. In Table II in which the positives for each test are given it may be seen that the total percentages obtained for the Kolmer and Kline are identical, while the Kahn is slightly lower. Eller and Rem reported the Kline diagnostic test more sensitive than the Kahn. In our series the Kahn was more sensitive in treated cases, but the Kline more sensitive in untreated ones.

In a few instances only were we able to carry out repeated Kline and sugar tests. Of 71 spinal fluids which gave Kline reactions of + to 4+, there was no

TABLE II  
SHOWING THE PER CENT POSITIVES OF THE KOLMER, KAHN AND KLINE, RESPECTIVELY

	SUGAR	TREATED SYPHILIS (C N S)		UNTREATED SYPHILIS		NO HISTORY OF SYPHILIS		UNCLASSIFIED		TOTAL	
		%	no	%	no	%	no	%	no	%	no
Kolmer Positive	-	18.1	23	30.7	36	0.18	1	4.8	40	1632	17.5
	+	22.8	29	57.2	67			5.7	47		
	0	12.6	16	6.8	8	0.18	1	2.2	18		
Total % & No		53.5	127	94.8	117	0.4	562	12.7	826		
Kahn Positive	-	22.2	10	28.5	20	0.6	2	3.7	10	695	16.5
	+	31.1	14	55.7	39			6.2	17		
	0	1.2	1	1.4	1			1.10	3		
Total % & No		54.5	45	85.7	70	0.6	308	11.0	272		
Kline Positive	-	13.3	17	30.7	36	0.7	4	4.6	38	1632	17.5
	+	20.4	26	52.9	62	0.3	2	6.6	55		
	0	11.0	14	8.5	10			2.6	22		
Total % & No		40.9	127	92.3	117	1.1	562	13.9	826		



change in this test in 52 (73.2 per cent) after an interval of two to nineteen days, regardless of the sugar test being negative later in all. In 10 of these after inoculation of *B. coli* the sugar test became negative but there was no change in the Kline. On a second examination in 13 (18.3 per cent) of the 71, the reaction was less marked, i.e., 7 giving 4+ became 2-, 5, 4+ became +, and one, 2- became -. From a case giving a 4-, one specimen (1.4 per cent) became negative in five days. Four (5.6 per cent) giving a 2- became negative in eight to nineteen days and one specimen (1.4 per cent) giving a - became negative in four days. In 14 specimens which had no sugar on the first examination, there was no change in + to 4- Kline reactions.

#### CONCLUSIONS

In the series of tests carried out here the relation between the results of complement fixation reactions and the precipitation tests was very close in the groups containing sugar and the ones in which sugar was absent. Judging by our figures it would appear feasible to perform Kline tests on spinal fluid specimens in which the presence of sugar cannot be demonstrated, providing the fluids are not grossly contaminated. However, it is shown that in a few specimens in which repeated Kline tests were done, a change occurred in some. Whether this was due to the factor causing the disappearance of sugar is a point to be determined. It seems logical that if a specimen is suitable for the complement fixation test, it should be possible to carry out a precipitation test, since it is a well-known fact that the substance which is responsible for the fixation of complement is responsible for the formation of the precipitate. On the other hand, complement fixation and precipitation have quite different optimal conditions. However, judging by the close agreement of our results of complement fixation and precipitation on fluids with and without sugar, it does not appear that the presence or absence of sugar is a factor. In the study made here the Kline test seemed to be preferable to the Kahn as a routine test with the Kolmer, since it gave a higher percentage of positives than the Kahn in untreated clinically syphilitic cases, while the Kahn offered no significant advantage over the Kolmer in treated cases. The performance of the Kline being more expeditious than the Kahn and being more easily read, offers an advantage over the Kahn. However, the Kolmer test probably due to the fact that it is an ice box fixation test and, therefore, usually more sensitive in spinal fluids where often a small amount of reagin is present, generally appears to be superior to either precipitation test.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A KILDUFFE, M D , ABSTRACT EDITOR

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## **PREGNANCY, Simple Test for, Using Immature Female Albino Rats, Kelly, G L J A M A 100 1010, 1933**

Intraperitoneal injections of from 5 to 15 cc of urine from pregnant women will cause premature establishment of the vaginal orifice in the albino rat weighing from 30 to 40 gm, usually within from seventy two to eighty four hours

Intraperitoneal injections of the same amount of urine from nonpregnant women will not cause such premature opening of the vagina

Two animals should be used and the opening of the vaginas of both is read as positive, of one, as doubtful (the test should be repeated), of neither within ninety six hours, as negative

If the vagina is open and the orifice obscure, one may see it by holding the animal by the neck and shoulder region and pulling strongly on the base of the tail In this maneuver the abdomen should not be compressed

The main advantages are the simplicity of the test and the ease of reading the result

## **TUBERCULIN, Comparative Sensitiveness of the Pirquet and Intracutaneous Test, Aronson, J D, Zacks, D, and Poutas, J J Am Rev Tuber 27 465, 1933**

It was shown in a comparative study that the intracutaneous tuberculin test is more sensitive than the Pirquet test in determining the incidence of tuberculous infection, and that the results obtained by the two methods are not directly comparable

## **TRANSFUSION Technic for Direct Matching of Blood, Simpson, J C Am J Clin Path 3 256, 1933**

1 Draw a drop of blood from patient's finger tip to the 1 mark in a leukocyte counting pipette and follow with 5 per cent sodium citrate solution to the 11 mark

2 Mix thoroughly by shaking and transfer to small glass vial suitably marked for identification

3 Repeat for each donor

4 With an erythrocyte pipette, make a 9 to 1 combination of patient's and donor's blood by drawing blood from donor's vial to the first or 01 mark and patient's to the 1 mark at top of lower portion of the pipette

5 Transfer mixture to end of a glass slide and mix by alternate sucking and blowing three or four times Add a small drop of saline and cover with cover slip

6 Now make a 1:1 dilution by drawing patient's blood from the vial to the 05 mark and donor's blood to the 1 mark Mix and transfer to the other end of slide in same manner

7 Prepare a separate slide in this way for each donor There is then a mixture of 9 parts of patient's blood to 1 of donor's at one end of slide and a 1 to 1 mixture at other end

8 Agglutination will show up both micro and macroscopically in two or three minutes at room temperature

9 If no clumping occurs in fifteen minutes transfusion is safe

10 Transfusion is dangerous if agglutination is present in the 9 to 1 combination

## ABSTRACTS

11 If the 9 to 1 mixture is clear and the 1 to 1 shows agglutination, transfusion can be done but with caution, be ready to stop it once if untoward symptoms develop

**TISSUE** The Adaptation of Formalin Fixed Tissue for Mallory's & Wright's Staining Methods, Kernohan, J W *Am J Clin Path* 3 255, 1933

The formalin fixed material is washed briefly in running water or ammonia water (10 cc of tap water and approximately 60 drops of ammonia), although both these procedures may be omitted. If there is much blood in the tissue, the ammonia water reduces the formalin precipitate on the stained preparations. The tissue is fixed in Zenker's solution for six to eight hours, and washed in running water for twenty minutes to half an hour. The tissue is placed overnight or longer in Weigert's secondary mordant for myelin sheath stains. It is then embedded in paraffin and cut and stained in the usual way with any of Mallory's stains.

**SCARLET FEVER** The Addis Sediment Count In, Lyttle, J D *J Clin Invest* 12 95, 1933

The technique of Addis for the quantitative mensuration of protein, casts and cells in the urine has been applied to 14 cases of scarlet fever. All cases showed moderate transient increases in the excretion of protein and formed elements during the period from eight to forty five days after onset of scarlet fever.

**URINE** The Addis Sediment Count in Normal Children, Lyttle, J D *J Clin Invest* 12 87, 1933

The following is suggested as a tentative standard in normal children four to twelve years old

	35 mg
Protein	10,000
Casts	600,000
Red blood cells	600,000 (male)
Epithelial and white blood cells	1,000,000 (female)

**TUBERCULOSIS** Studies in the Pathogenesis of Primary II Tendencies in Adult Tuberculous Infection, Sweany, H C, *Am Rev Tuberc* 27 575, 1933

A report has been made of 6 primary like infections in adult individuals that reveal a difference from the usual type in that the lymph node complex was very small or entirely lacking. The variation is so constant in adult people living in civilized communities that it raises the question of whether it is not the rule in such individuals. A few hints are made toward a more rational pathologic classification of primary tuberculous lesions than is represented by existing theories.

**TUBERCULOSIS** Studies on the Pathogenesis of Primary I The Regressive Lesion, Sweany, H C *Am Rev Tuberc* 27 559, 1933

A study of the regression of the primary tubercle has been made, based on the findings in 188 pairs of lungs removed at necropsy, radiographed, and sectioned by a special method. The incidence and location of these lesions were found in keeping with the ideas of Kuss, Ghon and others, but there was a higher incidence of what appeared to be multiple primary residues. This was thought to be due to a more certain method of localization. Of 88 specimens of characteristic primary lesions 76 were in the parenchyma and 65 in the lymphatics. Twenty three were located only in the parenchyma and 11 only in the lymph nodes.

In 84 calcified parenchymal lesions bone was found in 22, while in 97 lymph nodes bone was found in 23, revealing practically no difference in bone formation between the local and lymphatic lesion and an incidence of 25.1 per cent of all without the usual lymph node exaggeration. The mechanism of calcification, ossification and resorption are described.

The question arose regarding the possibility of the liberation of tubercle bacilli as a result of the resorption of the walls of the primary lesion, because walls were entirely gone in more relatively young individuals (one seventeen years old) while bacilli were living in most large primary lesions in people under twenty five years of age

Approximately half of the calcified parenchymal lesions may be seen on antemortem radiographs, for, out of 98 showing on the postmortem pictures, only 48 could be seen on the antemortem films. Ten contained very small primary lesions and thirty none at all. Many of these two groups showed a modified type of primary lesion, indicating that there had been only a partial sensitizing effect from the first infection. Six appeared to be adult primary lesions.

#### WIDAL REACTION The Bacteriology and Immunology of Enteric Fevers, Pijper, A South African M J 8 283, 1933

The author relies upon the complement fixation reaction for the early recognition of typhoid fever in preference to the Widal reaction the shortcomings of which he discusses.

He calls attention to the presence of and difference between H (flaky) agglutinins and O (dense, compact flakes) agglutinins and their differing clinical significance and also to the fact that O agglutinins can only be demonstrated when agglutination tests are made upon living organisms or upon alcoholic suspensions.

He formulates the following rules:

1 The Widal reaction, as commonly done with killed suspensions, elicits only H agglutinins and does not demonstrate O agglutinins.

2 While typhoid patients may produce both H and O agglutinins, they all produce O agglutinins early in the disease. In many cases H agglutinins may never be produced or too late to be of diagnostic value.

3 O antibodies are the same antibodies as those producing complement fixation, hence either test may be used for their detection providing that the agglutination test is carried out in accordance with the data above presented.

4 Typhoid vaccination causes the production of H agglutinins which may show a non-specific increase in the presence of febrile reactions whereas O agglutinins increase in titre only when the patient has typhoid.

5 H antibodies are valueless as a means of defense. O antibodies are the real immune bodies.

From these premises the following deductions follow:

a The serologic diagnosis of typhoid depends upon the demonstration of O antibodies by complement fixation or suitable agglutination tests.

b The strength of the positive reaction, as it depends upon the presence of truly defensive antibodies, is of some prognostic value. In this connection the test is best correlated with the blood culture.

c Carriers frequently do not show H agglutinins but always show O agglutinins and may be thus recognized.

d Oral immunization (Besredka method) causes the production of O antibodies.

#### DIPHTHERIA Replacement of Toxin Antitoxin by Toxoid With a Consideration of the Comparative Dosage, Park, W H Am J Pub Health 23 600, 1933

The conclusions advanced follow:

The manufacturers should be required to state on their labels the number of flocculation (antigenic) units in the amount in the syringe or vial, or the number of units per cubic centimeter.

Two doses of from 5 to 10 antigenic units each are sufficient to immunize from 90 per cent to 98 per cent of young children, and of a somewhat less percentage of older children. Even 2 doses of 2.5 units each will give us as much immunity as 3 doses of 1 cc of standard toxin antitoxin. If by refining we are able to obtain a much stronger toxin which does not produce too much local irritation we may use one dose only.

Toxoid has an advantage over toxin antitoxin in being much more stable

An interval of two weeks, or longer, is advisable between injections, but where it is much more convenient to make the interval only a week it is proper to do so

At any age after three months, infants respond well to injections of toxoid The choice time in the country is probably at six months, or a little later, and in the city, nine months or a little earlier The difference in the most desirable age is due to the fact that in the country a smaller percentage of infants obtain passive immunity from the mothers than in the city

**RHEUMATIC FEVER, Blood Cultures in Children, Wilson, M G., and Edmond, H Am J Dis Child 45 1237, 1933**

A total of 536 blood cultures were sent to the laboratory for study One hundred and forty seven (26 per cent) were found to be contaminated (137 on the first and 10 on subsequent openings) and were therefore discarded Of 389 blood cultures, 293 (75 per cent) remained sterile for the four week period of study Ninety six (25 per cent) of the cultures were considered positive

Thirty two of the uninoculated cultures were sent to the laboratory (unidentified) and subjected to identical bacteriologic examination Two, or 6 per cent, were found to be contaminated and were discarded Ninety four per cent remained sterile throughout the period of study

Two hundred and thirty six blood cultures from 67 children with rheumatic disease were studied In 46 per cent of these children, positive cultures were secured Positive cultures were obtained in 37 per cent during the active stage and in 41 per cent during an apparently inactive period

In the control series (which included normal infants and children as well as those who were ill or were convalescing from various infections), 153 blood cultures from 78 children and 13 adults were studied Forty one per cent of these cultures were positive Positive cultures were secured in 33 per cent of the healthy infants and children of this control group

The microorganisms recovered from all of the blood cultures were of 3 types, *Streptococcus viridans*, *Streptococcus anhemolyticus* and pleomorphic bacilli About one half of the organisms recovered were streptococci, the remainder were pleomorphic bacilli

The incidence of recovery of these organisms from both the rheumatic and the control series were comparable

The incubation period varied from three to thirty two days About one half of the strains (*streptococci* and *pleomorphic bacilli*) were recovered within a two week period of incubation

**SPIROCHETA PALLIDA, Modification of Dieterle Method for Single Sections, Krajian, A. A Am J Syph 17 127, 1933**

1 Fresh blocks of tissue about 5 mm thick are fixed in 10 per cent formalin at 70° C for ten minutes Of course, formal fixed tissue does not require this

2 Cut thin sections (5 to 7 microns) on the freezing microtome

3 Place the sections in the following solution for fifteen minutes at 70° C

Uranium nitrate	10 gm
Iormic acid, C P 85 per cent	30 cc
Glycerine	50 cc
Acetone	100 cc
Alcohol, 95 per cent	100 cc

4 Wash for a few seconds in distilled water

5 Place sections in a 0.75 per cent aqueous solution of silver nitrate for one hour at 70° C

6 Wash in distilled water for a few seconds

7 Place sections in 10 cc of the following developing solution for five to ten minutes, to which, just prior to use, one drop of albumin glycerin fixative is added and thoroughly mixed. The egg albumen glycerin fixative is that commonly used to fix paraffin sections to slides.

Hydroquinone	0.62 gm
Sodium sulphite	0.12 gm
Acetone	50 cc
Neutral formaldehyde, C. P. 40 per cent	50 cc
Pyridine	50 cc
Saturated solution of gum mastic in 95 per cent alcohol	50 cc
Distilled water	300.00 cc

8 Wash for a few seconds in distilled water.

9 Draw sections onto slides, dehydrate with absolute alcohol, clear in xylol, and mount in xylol gum damar.

#### PERTUSSIS, Medium for Isolation, Bailey, J. H. J. Infect. Dis. 52: 96, 1933

The ingredients are: potato 200 gm., potato starch, 18 gm., chemically pure glycerin, 30 cc., and distilled water, 600 cc.

The potatoes are pared and cut into thin slices. A quantity of potato slices weighing 200 gm. is placed in a tared double boiler along with the potato starch, glycerin and water. Potato flour may be used in place of the potato starch. The mass is then cooked for two hours, being stirred constantly until it thickens, then only occasionally. Thickening usually occurs within ten minutes. At the end of two hours the water lost by evaporation is replaced, and to each 1 cc. of starch mass is added 3 cc. of 0.6 per cent sodium chloride solution. The mixture is shaken thoroughly and then filtered through four layers of cheesecloth. This filtration is rapid. The filtrate is then distributed, in 200 cc. lots, into flasks of convenient size (500 to 750 cc.) and 10 gm. of agar added to each flask. The flasks are plugged and sterilized in the autoclave of twenty minutes at 15 pounds (8.3 kg.) of pressure. This forms the stock base of the medium, and may be stored in the refrigerator until needed. To complete the medium, a flask of the base is melted, preferably in the autoclave, and when the melted medium has cooled to about 40° C., 200 cc. of sterile defibrinated horse blood is added and thoroughly mixed. Then 6 cc. of sterile half-normal lactic acid is added and thoroughly mixed, care being used to prevent frothing of the medium. The medium may now be poured into petri dishes or tubes.

A medium for the isolation of *B. pertussis* should be a bright red to give optimum results. This fact has not received the emphasis due it.

#### SYPHILIS, Comparison of Results Obtained With Kahn, Kline, Hinton, Meinicke, Sachs-Georgi, and Rosenthal Tests for, Burdon, K. L., and Duggan, L. B. Am. J. Syph. 17: 110, 1933

The results of Kline, Kahn, Hinton, Sachs-Georgi, Meinicke, and Rosenthal tests performed at the same time on the sera from 400 individuals indicate that the Kline, Rosenthal, and Meinicke methods are the most sensitive, that the Hinton and Kahn tests are somewhat less so, and that the Sachs-Georgi test is comparatively insensitive. With the sera from 80 patients with late or latent syphilis the Sachs-Georgi method was positive in no more than 6 per cent, while positive reactions were recorded with the Kahn test in 24 per cent, the Hinton in 28 per cent, the Kline in 56 per cent, the Meinicke in 58 per cent, and the Rosenthal in 61 per cent.

Comparison of the results of parallel tests by the Kline and Kahn methods on 1235 specimens confirmed the greater sensitivity of the Kline over the standard Kahn test. The latter gave a reaction of slightly greater degree in only 7 cases (0.5 per cent). On the other hand, in approximately 14 per cent of the tests the Kline reaction was doubtful ( $\pm$  or  $+$ ) while the Kahn was negative, or definitely positive ( $++$ ,  $+++$ , or  $++++$ ) while

the Kahn was doubtful, and in another 36 per cent the Kline test was positive and the Kahn entirely negative.

In the great majority of instances the reactions of the more sensitive tests were justified by the known existence of syphilitic infection in the patients. False positive reactions were not observed with any of the methods except with the Kline test, and then only in 2 instances, when the "very sensitive" antigen emulsion was used. A few nonspecific doubtful (+ or -) reactions in normal sera were recorded, however, with all of the methods, except the Sachs-Georgi test. These reactions were observed most frequently with the Rosenthal test, but the number recorded rapidly declined as experience with the method increased. The next largest number were observed with the Kline test, doubtful reactions being recorded in about 3 per cent of the 227 normal sera tested. Less than 1 per cent of the Kahn, Hinton, and Meinicke tests give these reactions.

Since all of the tests have been developed empirically, and none are, in a strict sense, specific for syphilis, there is no absolute standard by which their merits may be judged. They are all more or less efficient indicators of the same change in the serum which is the characteristic sign of syphilitic infection. The methods differ considerably in sensitivity when a certain particular technique prescribed by their originators is followed, as in the present series of tests. It must be realized, however, that the degree of sensitivity shown by a certain method in such a comparative study is not a fixed or constant attribute. Without any fundamental changes in the technique any one of the methods may be adjusted as to be more or less sensitive. It may be remarked parenthetically that the sensitivity of complement fixation tests is also subject to wide variation, and the statements sometimes seen in the literature to the effect that this or that precipitation test is more or less sensitive than "the" Wassermann test are utterly pointless. Among the precipitation tests the most useful are those which are most readily adjusted to different degrees of delicacy, and which give clear cut reactions, easily controlled, throughout a wide range of sensitivity. With such a test, in the hands of a competent serologist who understands thoroughly the factors influencing that particular method, a really adequate serologic study of cases showing borderline reactions could be made, and these cases constitute the only real problem. Economy of time and materials and ease of preparing reagents are further qualities to be desired. Thus, in appraising the various tests, their general practicability is after all a primary consideration.

With this in mind the authors believe the Kline and Kahn methods are to be especially commended, and that the Hinton test is also acceptable, whereas the Sachs-Georgi and Meinicke methods are not satisfactory. When the Rosenthal test is conducted according to the new procedure described, it appears to be of practical value as a supplemental microscopic test, particularly in laboratories where Kahn tests are done routinely.

The authors' results, as well as those of many other workers, show that no two methods can be expected to yield identical reactions in all sera. Nothing is to be gained, however, by performing a multiplicity of different tests on every serum. It would seem that the most desirable arrangement would be to carry out first a simple, rapid, presumptive test (conveniently a microscopic method), so adjusted as to be highly sensitive, such as the Kline test. This would result in the elimination from further consideration of all the frankly negative specimens which commonly comprise as much as 80 per cent of all (the use of a presumptive form of the Wassermann test for the same purpose is as long ago recommended). The same test in less sensitive form, or perhaps preferably another well understood method, either a complement fixation procedure or a test tube precipitation test, may then be performed on the remaining sera. Those specimens showing borderline reactions should be subjected to still further testing by one of the methods in quantitative form.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughn, Professional Building, Richmond, Va

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### Asthma, Hay Fever and Related Disorders

THIS is a very readable primer on allergy for the use of the patient, a perusal of which will give him an understanding of how allergy behaves and what the allergist is attempting to accomplish. It is not a guide to treatment in that there are no detailed instructions to be used in this or that case. It is, in effect, just what the author intended, an explanatory handbook of just what we are talking about when we discuss allergy.

### Diseases of the Heart

SINCE Sir Thomas Lewis' preeminence as a cardiologist is known by all, the value of the book under review may be taken for granted. Those who have read his earlier volumes realize the conciseness of his manner of presentation, the absence of paraphrasing and superfluity.

The present volume to an extent represents a milestone in the published volumes of Sir Thomas Lewis. First, his style while in no sense verbose is freer and more elastic. Second, there is a minimum of presentation of protocols and experimental observation. Third, and this is the real reason, he is writing the book for the general practitioner of medicine. It is not a report of experimental studies but a reference volume on the treatment of the general run of cardiac diseases. The reader will not be baffled by finding that he must take an electrocardiographic tracing of each case or make special laboratory studies. These are of utmost value in cardiac research and often of great value in clinical studies, but more often entirely unnecessary.

The author's arrangement is original and most logical. The first section deals with cardiac failure in its various phases, the second with coronary thrombosis, the third with angina, the fourth with changes of rate and rhythm, and this is followed by discussions of valvular disease, effort syndrome, pulmonary edema, pericarditis, infection, hypertension, arteriosclerosis, congenital lesions, etc.

Scattered through the volume are statements which one could almost term aphorisms which the author has derived from his own experience and which we almost wish he had italicized. The following are examples:

"Cardiac failure may be divided into two stages, the stage of symptoms or warning reserve and the stage of signs or of circulatory embarrassment and breakdown."

"It is to be realized that by the time congestion of the venous system sets in, nine tenths of the heart's capacity to perform its task has been lost."

"Of the very numerous tests of cardiac efficiency there is none that approaches in delicacy the symptom of breathlessness."

"It is generally redundant to set up deliberate exercise tests."

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\*Asthma, Hay Fever and Related Disorders. A Guide for Patients. By Samuel M. Feinberg, M.D., F.A.C.P., Assistant Professor of Medicine and Attending Physician in Asthma and Hay Fever Clinic, Northwestern University Medical School. Attending Physician, Cook County Hospital, Chicago. Cloth, pages 121, illustrated. Lea and Febiger, Philadelphia, 1933.

†Diseases of the Heart. Described for Practitioners and Students. By Sir Thomas Lewis, C.B.E., F.R.S., M.D., D.Sc., LL.D., F.R.C.P., Hon. D.Sc. (Michigan), Physician in charge of Department of Clinical Research, University College Hospital, London. Physician of the Staff of the Medical Research Council. Physician in chief (pro tem), Peter Bent Brigham Hospital, Boston. Honorary Fellow, New York Academy of Medicine. Corresponding Member, Association of American Physicians and Interstate Postgraduate Medical Association. Cloth, pages 297. The Macmillan Company, New York, 1933.



"A comparison between a healthy and an unhealthy individual is usually far less satisfactory than between the healthy and unhealthy states of the same subject"

"In studying cardiac failure at certain stages, the signs to watch are not in the heart, but in the veins and the liver, in the legs and at the bases of the lungs"

## American and Canadian Hospitals

THIS reference volume takes its place alongside of the Directory of the American Medical Association and Finkeld's "American Physicians and Surgeons". The editor has developed it with the cooperation and endorsement of the American Hospital Association and other various interested associations. The first 80 pages contain brief historical descriptions by various contributors, of the development of the various hospital associations such as the American Hospital Association, the Canadian Hospital Council, the American College of Surgeons, the Catholic Hospital Association, the American Nurses' Association, the American Occupational Therapy Association, etc. The major portion of the 1500 pages is taken up with descriptive information of hospitals in the United States and Canada, including even such remote sections as Alaska, Guam, Hawaii, the Philippine Islands, Labrador, Newfoundland, and Yukon.

The list is not selected but includes all hospitals, even small hospitals in the smallest villages with no more than five beds. Information available includes description of types of cases admitted and not admitted, special departments, ownership, whether approved by American College of Surgeons, whether approved by the A M A for internship, whether a member of the American Hospital Association, size, cost, rates, annual statistical information on admittances, and officers.

This is a volume that should be had by all hospitals, by business enterprises trading with hospitals, and should be available for physicians to consult in selecting the proper hospital to which to send their patients.

## A Standard Classified Nomenclature of Disease†

THE need for a standardized nomenclature acceptable to all physicians has been strongly felt for many years. Classifications such as those used at Bellevue Hospital, the Peter Bent Brigham and associated hospitals and others have been acceptable but have not been uniform. Since there is rather constant need for revision and since these revisions should where possible correlate the various classifications, a conference on nomenclature of disease was held in New York on March 22, 1928, upon the invitation of the New York Academy of Medicine. From this developed a large committee entitled "The National Conference on Nomenclature of Disease" headed by Haven Emerson, Henry Christian, and E H L Corwin and having representatives from a large number of the leading medical societies in the country.

The outcome of the work of the conference is the present volume. It has been approved by all of the cooperating organizations and hospitals and will undoubtedly be from now on the recognized standard. The volume is not large and is so thoroughly indexed that no physician will have any difficulty in adapting his diagnoses to the standard nomenclature. The advantages of such a procedure are obvious and it is hoped that all hospitals will henceforth require that diagnoses be recorded in accordance with this nomenclature and that all physicians who make any attempt at keeping cross files by diagnosis will adopt it.

\* American and Canadian Hospitals. A Reference Book giving Historical Statistical and other information on the Hospitals and Allied Institutions of the United States and Possessions and the Dominion of Canada. Edited by James Clark Finkeld with the co operation of the American Hospital Association. Cloth pages 1560. Midwest Publishers Company Minneapolis 1933.

† A Standard Classified Nomenclature of Disease. Compiled by The National Conference on Nomenclature of Disease. Edited by H B Logie MD. C M Executive Secretary. Cloth pages 702. The Commonwealth Fund New York 1933.

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## EDITORIAL

### Liver and Brain—Pathologic Interrelationships

THERE has been, shall we say, a superstition through the ages that the liver, under certain circumstances, exerts a direct action upon the mind or the brain. That the supposed action is a deleterious one is indicated by such terms of ancient usage as "choleeric" and "hypochondriac." In more recent years the possibility of recognizable organic damage to the central nervous system due to or associated with liver pathology has aroused more serious interest. The most clear-cut example is the occurrence of hepatic cirrhosis with degeneration of the lenticular nucleus, described by Wilson in 1912. Many instances have since been reported of comparable coincidences in which hepatic disease preceded nervous manifestations and in which at autopsy spongy degeneration of the corpus striatum with proliferation of blood vessels and glia cells, was demonstrated. Some of these cases have presented symptoms

suggestive of Parkinson's disease, others have resembled encephalitis. Occasionally there has been an associated combined degeneration of the spinal cord.

Explanatory theories have suggested that liver disease was the primary factor, with secondary toxic action on the nervous system, or that both the hepatic and central nervous pathology were due to some intoxication from another source within the body, that the combined occurrence of disease in both tissues represented some congenital deficiency, or that the original source of trouble was in the central ganglia regulating hepatic circulation, disease of which produced functional disturbances in the liver.

Crandall and Cherry have recently observed that in individuals suffering from hepatic disease there is a decided increase in the oil-splitting lipase in the serum. This was also observed in patients suffering from multiple sclerosis. Crandall and Weil have recently reported further experimental investigations in this regard.

These authors have produced liver damage in dogs by either of three methods, ligation of the common bile duct, ligation of the pancreatic duct, and by an Eck fistula. They have also produced liver damage in rats by ligation of the common bile duct. Ligation of the pancreatic duct or bile duct produces a severe fatty degeneration of the liver. The degeneration is not as severe in rats.

The blood serum of dogs with liver damage produced by these methods contains a substance, appearing on and after the fourth day, which exerts a destructive action on the spinal cords of rats, *in vitro*. There results a pronounced swelling and fragmentation of the nerve fibers with demyelination, destruction of glia nuclei and, apparently, granular disintegration of the nerve cells. This appears not to be due to the increased lipase content since after several days the lipase level fell while the toxic action persisted or became more pronounced. The authors succeeded in separating the toxic substance from the proteins of the blood.

They next studied changes produced in the nervous structures of dogs and rats which had been subjected to the above procedures and which were examined at intervals from ten days to six months after survival from the operation. In dogs they found, in general, spongy necrosis in the walls of the lateral ventricles with foci of edema in the white matter and extensive glia proliferation. Severe damage was usually found in the choroid plexes. This with the spongy degeneration of the ventricular walls suggested that the toxic substances were eliminated into the cerebrospinal fluid. The extent of damage decreased with increasing distance from the ventricles so that at more remote areas only nerve cell degeneration and glia proliferation were observed. Damage to the striate body was frequently seen.

In one dog and more consistently in the rats, another mode of elimination of toxic substances seemed to have occurred. In these, ventricular and choroid lesions were not observed but instead scattered foci of demyelination around the blood vessels with overgrowth of fibrous glia suggesting elimination through the vessel walls. Perivascular hemorrhages were observed early in this type of reaction.

Crandall and Weil point out the similarity of many of their pathologic findings to those observed in cases of Wilson's disease, the common features being particularly spongy necrosis of nerve tissue, active proliferation of glia with the formation of large round or oval nuclei, diffuse nerve cell disease with predilection for the deep cortical layers and the striate body, with absence of active mesenchymal reaction. At the same time they point out that with the varied distribution and the character of the lesions, the same toxic factor can very readily produce a variety of combinations of pathologic lesions with a resultant distinct variability in symptomatology. "Such a point of view will prevent one from expecting stereotype encephalopathies in hepatic disease, or from assuming dogmatically one and the same etiology for all cases of hepatolenticular degeneration."

They conclude that experimental observations of hepatocerebral interrelationships such as those described will be more successful in demonstrating such relationships than clinical-pathologic observations since complicating factors such as arteriosclerosis and chronic focal infection which so often accompany hepatic and central nervous disease are thereby eliminated.

The observations of these investigators contribute further experimental corroboration of a phenomenon which has long been recognized clinically.

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